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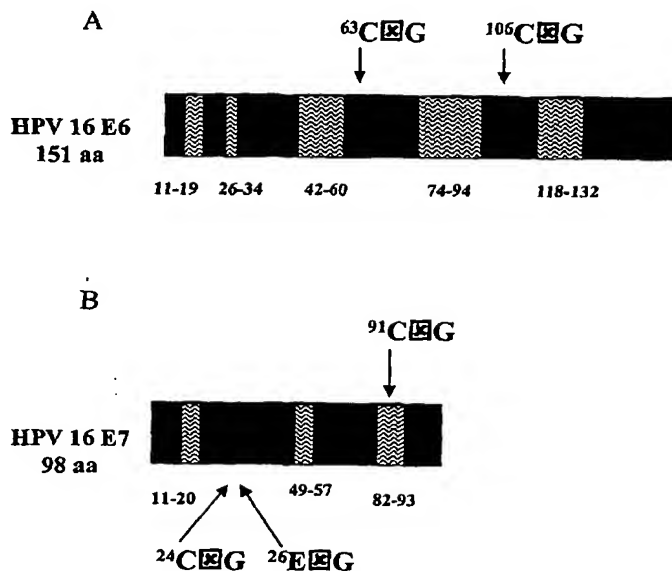
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(57) Abstract: The present invention provides immunogenic and pharmaceutical compositions for the treatment and prevention of human papillomavirus (HPV)-associated cancers and in particular, cervical cancer. In particular, this invention relates to fusion proteins, and the nucleic acids encoding these fusion proteins, used to generate immune responses against HPV. Specifically, this invention provides for fusions of HPV E6 and E7 in which the E6 and/or E7 contains one or more mutations. These mutations abrogate the transformation activity of these oncogenic proteins and, thus, confer safety to the E6/E7 fusions. In addition, these fusions maintain or increase the immunogenic efficacy of E6 and E7. Any gene or protein delivery method can be used to deliver or package the immunogenic compositions of the present invention.

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**HUMAN PAPILLOMAVIRUS POLYPEPTIDES AND IMMUNOGENIC COMPOSITIONS**

This application claims priority to U.S. Provisional Patent Application Serial No. 60/415,929, filed October 3, 2002, which is incorporated herein by reference in its entirety.

**FIELD OF THE INVENTION**

This invention relates to pharmaceutical and immunogenic compositions used to treat or prevent cervical cancer and other cancers caused by human papillomaviruses (HPV). In particular, this invention relates to fusion proteins, and the nucleic acids encoding these fusion proteins, used to generate immune responses against HPV. These fusion proteins and polynucleotides are used in the treatment and prevention of HPV-induced cancers.

**BACKGROUND OF THE INVENTION**

Cancer of the uterine cervix is the second leading cause of tumor-related deaths in women, accounting for 250,000 deaths per year worldwide. Greater than 99% of all cervical cancers are known to be associated with human papillomavirus (HPV) infection, of which 50% are directly linked to HPV type 16 (HPV16) (Walboomers et al., J. Path. 1999, 189:12-19). While the majority of HPV16 infections are asymptomatic and transient, a certain percentage of them become persistent. About 1% of persistently infected individuals progress through increasingly severe cervical lesions known as cervical intraepithelial neoplasia (CIN), and eventually to invasive cervical carcinoma.

The early HPV proteins known as E6 and E7 are required to maintain the malignant phenotype (Von Knebel et al., Int J Cancer 1992, 51:831-4; Crook et al., Embo J 1989, 8:513-9; He and Huang, Cancer Res. 1997, 57:3993-9). These proteins are consistently expressed in CIN lesions and cancers (Smotkin and Wettstein, Proc Natl Acad Sci USA 1986, 83:4680-4; Durst et al., Virology 1992, 189:132-40). These proteins induce proliferation of the epithelium by disrupting the regulation of the cell cycle. Specifically, E7 binds and inactivates the cellular

tumor suppressor retinoblastoma protein (Rb) (Dyson et al., Science, 243:934-7), which results in progression of the cell into S-phase of the cells cycle (Cobrinik et al., Trends Biochem Sci 1992, 17:312-5). The E6 protein of HPV16 induces degradation of the tumor suppression protein p53 (Scheffner et al., Cell 1990, 63:1129-36), preventing the cell from undergoing apoptosis.

Because tumor cells constitutively express the E6 and E7 proteins, and these proteins are not present in normal cells, these viral proteins are very attractive targets for cancer immune therapy. Many lines of evidence suggest that a cellular-mediated immune response against E6 and E7 in humans correlates with the natural regression of HPV lesions and viral DNA clearance (Nakagawa et al., J Infect Dis 1997, 175:927-31; Kadish et al., J Natl Cancer Inst 1997, 89:1285-93). Also a CTL response against E7 has been shown to protect mice against HPV16-positive tumors in different murine models (Feltkamp et al., Eur J Immunol 1995, 25:2638-42; Lin et al., Cancer Res 1996, 56:21-6).

Since cell-mediated immunity (CMI) appears important in controlling HPV infection and disease (Eiben, G.L. et al., Adv Can Res 2002, 86:113-148), a therapeutic vaccine should generate optimal T cell responses against numerous HPV E6 and E7 antigenic peptides for effective coverage in human leukocyte antigen (HLA) diverse populations.

A promising vaccine vector for delivering E6/E7 antigens is a recombinant alphavirus (AV) vector derived from the attenuated 3014 strain of Venezuelan equine encephalitis virus (VEE; Velders M.P. et al., Cancer Res 2001, 61:7861-7867). Replication incompetent VEE replicon particles (VRP) have proven to be highly effective vaccines in a number of preclinical infectious disease and tumor models (Rayner, J.O. et al., Rev Med Virol 2002, 12:279-296). AV-derived replicon vectors such as VEE encode heterologous genes in RNA form, do not spread beyond initial infection, and induce apoptosis of infected cells (Griffith, D.E. et al., Annu. Rev. Microbiol. 1997, 51:565-592). These attributes limit the opportunities for either prolonged protein expression or integration into host DNA which are characteristics of HPV-induced malignancies following natural infection. The low prevalence of pre-existing anti-VEE immunity and the prospects for repeated immunization with VRPs (Pushko, P. et al., Virology 1997, 239:389-401) are advantages over other recombinant viral vectors such as vaccinia virus or adenovirus.

throughout and have targets for cancer immune therapy, E6 and E7 have transforming activity. Consequently, immunotherapy methods using E6 and E7 that are currently contemplated in the art are potentially risky because these proteins can induce transformation and immortalization of cells.

5 There remains an unfulfilled need for efficacious compositions for the treatment and prevention of CIN and cervical carcinoma. More specifically, there is a need for immunogenic compositions, including E6- and/or E7-based compositions, that are both safe and effective for treating and/or preventing CIN, cervical carcinoma, anal carcinoma, and other such disorders.

10 Previous studies of HPV immunogenic compositions have been limited by the lack of HLA class I expressing tumor models in mice. An HPV16 E6/E7 positive model is described herein; this model forms progressively growing tumors in HLA-A\*0201 transgenic mice.

### SUMMARY OF THE INVENTION

15 The present invention fulfills the above described and other needs by providing pharmaceutical compositions and polypeptides comprising fusions of E6 and E7 polypeptides bearing mutations and/or a fusion order (E7E6) that decreases the biological activity and therefore transforming activity potential of E6 and E7.

20 In particular, the present invention provides novel E6/E7 fusion polypeptides and the polynucleotides that encode them. In specific embodiments, the invention provides polypeptides comprising the human papillomavirus E6 and E7 polypeptides wherein the E7 polypeptide has mutations at any one or more of the amino acids corresponding to amino acids 24, 26 or 91 of SEQ ID NO: 14 and the E6 polypeptide has no mutations or has mutations at any one or more amino acids corresponding to amino acids 63 or 106 of SEQ ID NO: 13. In a specific embodiment, the fusion polypeptide comprises an E7 polypeptide in which one, or preferably both, of the amino acids corresponding to amino acids 24 and 26 of SEQ ID NO: 14 are mutated. 25 These mutated polypeptides may have a glycine residue at any of these mutated positions and E6 may be carboxy terminal or amino terminal to E7.

The present invention also provides for isolated nucleic acids that encode these polypeptides. For example, in one embodiment of the invention the isolated nucleic acids comprise human papillomavirus E6 and E7 sequences wherein the E6 nucleotide sequence has



mutations at any nucleotide(s) corresponding to nucleotides 187-189 of the HPV 16 E6 gene, and the E7 nucleotide sequence has mutations at any nucleotide(s) corresponding to nucleotides 70-72, 76-78 or 271-273 of the HPV 16 E7 gene, and wherein the mutation or mutations result in a different amino acid being encoded for. The present invention also provides pharmaceutical and immunogenic compositions comprising these polypeptides and polynucleotides.

The present invention also provides for isolated polypeptides having the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, or SEQ ID NO: 11. In other embodiments, the invention provides isolated nucleic acids that encode such polypeptides, including nucleic acids having the nucleotide sequence SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 10 or SEQ ID NO: 12.

Expression vectors comprising the nucleic acid sequences encoding E6/E7 fusions are also provided. For example, in a specific embodiment, the invention provides expression vectors comprising SEQ ID NOS: 4, 6, 10 or 12 operatively associated with (e.g., under the control of) an expression control sequence.

Host cells comprising nucleic acids encoding E6/E7 fusions are also provided, as well as host cells that express or contain the E6/E7 fusion polypeptides.

In still other embodiments, the invention also provides immunogenic compositions that comprise a nucleic acid or polypeptide of the invention and are useful, e.g., for treating or preventing cervical cancer. Such immunogenic compositions may comprise (a) a polypeptide of the invention (e.g., a polypeptide comprising the human papillomavirus E6 and E7 polypeptides wherein the E7 polypeptide has mutations at any one or more of the amino acids corresponding to amino acids 24, 26 or 91 of SEQ ID NO: 14 and the E6 polypeptide has no mutations or has mutations at any one or more amino acids corresponding to amino acids 63 or 106 of SEQ ID NO: 13), and (b) a pharmaceutically acceptable carrier. Optionally, an immunogenic composition of the invention may also contain an adjuvant.

Recombinant viruses are also provided that contain one or more nucleic acids of the invention, and/or encode one or more of the invention's polypeptides. Thus, for example, a recombinant virus of the invention may comprise a nucleic acid encoding a polypeptide as set forth in SEQ ID NOS: 3, 5, 9 or 11, and more particularly having the sequence set forth in any of

SEQ ID NOS: 4, 6, 10 or 12. In a particularly preferred embodiment, the recombinant virus of the invention is a modified Venezuelan equine encephalitis virus (VEEV).

Methods of using the above-mentioned compositions are additionally provided, and such methods are also considered part of the present invention. Thus, the invention also provides a method for producing an immune response in an individual by administering to that individual immunologically effective amounts of a polypeptide of the invention (e.g., one having the amino acid sequence set forth in any of SEQ ID NOS: 3, 5, 9 or 11) and a pharmaceutically effective carrier.

Methods for treating and/or preventing cervical cancer are also provided. For example, in one embodiment the invention provides methods for treating cervical cancer in which a patient diagnosed with cervical cancer is administered a polypeptide of the invention (e.g., one having the amino acid sequence set forth in any of SEQ ID NOS: 3, 5, 9 or 11) and a pharmaceutically acceptable carrier. In other embodiments, the invention provides methods for preventing cervical cancer in which a polypeptide of the invention and a pharmaceutically acceptable carrier are administered to an individual. In still other embodiments, the invention provides methods for treating and/or preventing cervical cancer in a patient by administering to the patient an immunologically effective amount of a nucleic acid of the invention (e.g., one having a nucleic acid sequence encoding a polypeptide having an amino acid sequence of SEQ ID NOS: 3, 5, 9, or 11, particularly set forth in any of SEQ ID NOS: 4, 6, 10 or 12).

## BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are schematic diagrams showing the location of the point mutations introduced into HPV16 E6 and E7 proteins in relation to putative epitopes for the major HLA-A Class I alleles. Figure 1A shows the naturally occurring amino acids in E6, <sup>63</sup>C and <sup>106</sup>C, that were each mutated to glycine. Figure 1B shows the naturally occurring amino acids in E7, <sup>24</sup>C, <sup>26</sup>E, and <sup>91</sup>C, that were each mutated to glycine. Previously defined class I epitopes capable of binding HLA-A1, A2, A3, A11, and A24 alleles are shown in stippled boxes (Kast, W.M. et al., J Immunol 1994, 152:3904-3912).

Figures 2A-E are schematics showing the alignment and consensus sequence of the amino acid sequences of the E6 polypeptides of human papillomaviruses 18, 31, 33, 35, 39, 45,

51, 52, 56, 58, 59 and [REDACTED] SEQ ID NOS: 15-26, respectively). The consensus sequence (SEQ ID NO: 39) is shown below the alignment. Capitalized letters in the consensus sequence indicate complete consensus and lower-case letters in the consensus sequence indicate high-frequency, but not complete consensus.

5        Figures 3A-C are schematics showing the alignment and consensus sequence of the amino acid sequences of the E7 polypeptides of human papillomaviruses 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 (SEQ ID NOS: 27-38, respectively). The consensus sequence (SEQ ID NO: 40) is shown below the alignment. Capitalized letters in the consensus sequence indicate complete consensus and lower-case letters in the consensus sequence indicate high-frequency, but not complete consensus.

10        Figures 4A and 4B are graphs showing percentage specific lysis versus effector to target cell (E:T) ratio, which represents CTL responses following VRP immunization. C57BL/6 mice were immunized subcutaneously with  $3 \times 10^5$  IU of the indicated VRP, and CTL assays were performed 1 month later. Cytotoxicity was measured by Europium release of E7-MVA (A) or E6-MVA (B) infected MC57G target cells. These results were reproduced in two additional experiments (data not shown).

15        Figures 5A and 5B are graphs demonstrating percentage tumor free mice versus number of days after tumor challenge. C57BL/6 mice ( $n = 8/\text{gp}$ ) were primed and boosted with  $3 \times 10^5$  IU of the indicated VRP immunogenic composition on days -21 and -7 and challenged on day 0 with either  $5 \times 10^5$  C3 (A) or  $5 \times 10^4$  TC-1 (B) tumor cells in the flank. Tumors were monitored every 3 days.

20        Figure 6 is a graph demonstrating percentage tumor free mice versus number of days after tumor challenge. C57BL/6 mice ( $n = 8-16/\text{gp}$ ) received  $5 \times 10^5$  C3 tumor cells on day 0 and were immunized with the indicated VRP at days 7, 14, and 21. Tumors were monitored every 3 days over 45 days.

25        Figure 7 is a graph demonstrating percentage tumor free mice versus number of days after tumor challenge. HLA-A\*0201 transgenic mice ( $n = 10/\text{gp}$ ) received  $2 \times 10^6$  HLF16 tumor cells on day 0 and were immunized with the indicated VRP at days 5, 10, and 15. Tumors were monitored every 5 days.

Figures 8A and 8B are Western blots detecting p53 (A) and Rb (B) expression in primary human mammary epithelial cells (MECs) infected with different VRP preparations. Twenty-four hours following infection with the indicated VRP (at MOI = 10), 25 ug of each MEC cell lysate were run on SDS-PAGE, blotted, and probed for levels of p53 (A) and Rb (B). Equivalent protein loading was verified by probing with anti-tubulin antibody. The presence of E7 protein was verified by probing the indicated lanes with an anti-E7 antibody.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for fusions of HPV E6 and E7 proteins, the nucleotide sequences encoding examples of such fusions, and mutant forms thereof. Although each of the specific mutations of HPV16 identified herewith (with the exception of E6 C106G, which had only previously been mutated to C106R (Dalal et al., J Virol 1996, 70:683-8)) has been previously disclosed, combinations of these mutations have not been made and combinations of these mutations have not been tested for their ability to retain immunogenicity while lacking transforming or immortalization capacity. For example, it was not known whether any other combinations of two or more mutations would result in polypeptides that maintained their immunogenic efficacy. The present invention discloses fusions of E6/E7 (the term "E6/E7" is used herein to indicate fusions in the order E6E7, E7E6 or both) proteins containing unique combinations of these mutations and the surprising finding that E6/E7 fusion proteins containing four or five defined mutations maintain their immunogenic efficacy. This finding is particularly important because a therapeutic immunogenic composition should generate optimal cellular responses against numerous HPV E6 and E7 antigenic peptides for effective coverage in HLA diverse populations. The present invention also discloses that these E6/E7 fusions bearing multiple mutations, while maintaining their immunological efficacy, do not maintain the functions necessary for E6's and E7's transforming capacity, namely p53 and pRB degradation. Specifically, the fusions of the present invention do not induce degradation of p53 or Rb and, thus, are safer than their non-mutant counterparts for delivery into or expression in a patient.

The present invention also discloses the surprising finding that E7E6 fusions, that is fusions in which E6 is carboxy terminal to E7, have increased immunological activity as compared to their E6E7 counterparts.

In specific examples, HPV16 E6E7 and E7E6 fusion proteins were produced and tested for immunogenicity and anti-tumor responses. Several point mutations were introduced into the E6 and E7 genes to inactivate their oncogenic potential, while preserving known HLA epitopes. Comparable CTL responses to the H-2D<sup>b</sup> restricted E7<sub>49-57</sub> epitope were observed among mice immunized with 3 x 10<sup>5</sup> infectious units of VRPs encoding wildtype or mutant fusion proteins. All of the wt and mutant fusion protein-expressing VRP immunogenic compositions eradicated 7 day-established C3 tumors in 90% or more of mice. In addition, E6E7 fusion constructs demonstrated anti-tumor efficacy in two other E6E7-positive tumor models. Specifically, E7E6 TetM VRPs conferred complete tumor rejection in the HLF16 tumor model. Primary human mammary epithelial cells infected with VRPs expressing mutant, but not wildtype, E6 and E7 genes demonstrated normal levels of both p53 and retinoblastoma proteins.

#### **The E6E7 and E7E6 fusions of the invention**

The present invention provides for E6/E7 fusion polypeptides comprising multiple mutations, such as C24G, E26G and C91G in E7 and C63G and C106G in E6 (see Figures 1A and B). For example, the E6E7TetM and E7E6TetM fusion polypeptides comprise the E7 C24G and E26G mutations and the E6 C63G and C106G mutations, while the E6E7PentM and E7E6PentM fusion polypeptides comprise the C91G E7 mutation in addition to the four mutations present in the TetM mutants. These mutated fusion polypeptides, *inter alia*, may be unstable. Those of ordinary skill in the art appreciate that unstable proteins, as compared to stable proteins, have an increased capacity to develop CTL responses. Those skilled in the art will also appreciate that fusion proteins tend to not fold properly and thus are less stable than their non-fused counterparts. Thus, fusion proteins, such as those disclosed in the present invention, are better suited towards the production of cell-mediated immune responses than their unfused counterparts.

HPV16 E6E7 and E7E6 fusion proteins were produced and tested for immunogenicity and anti-tumor responses. Several point mutations were introduced into the E6 and E7 genes to inactivate their oncogenic potential, while preserving known HLA epitopes. Prior to the present invention it was not known whether the combinations of the mutations tested herein would destroy the polypeptides' immunogenicity or whether such mutated polypeptides would retain their immunogenicity. Comparable CTL responses to the H-2D<sup>b</sup> restricted E7<sub>49-57</sub> epitope were

observed among mice immunized with  $3 \times 10^7$  infectious units of VLP encoding wild type or mutant fusion proteins. All of the wild type and mutant fusion protein-expressing VRP immunogenic compositions eradicated 7 day-established C3 tumors in 90% or more of mice. In addition, E6/E7 fusion constructs demonstrated anti-tumor efficacy in two other E6E7-positive tumor models.

The present invention provides fusion polypeptides comprising E6 and E7, in which E6 is either at the amino terminus or at the carboxy terminus. However, those skilled in the art will recognize from the instant disclosure that fusions in which E6 is carboxy terminal to E7 (E7E6 fusions), that is E6 follows E7, such as E7E6TetM and E7E6PentM, will have increased immunological efficacy as compared to fusions in which E6 is amino terminal to E7 (E6E7 fusions, such as E6E7TetM and E6E7PentM). In addition, it will be recognized by those skilled in the art that E7E6 fusions, that is fusions in which E6 is carboxy terminal to E7, will have decreased E6 activity as compared to E6E7 fusions. Thus, E7E6 fusions will generally be expected to be safer. Accordingly, fusions in which E6 is carboxy terminal to E7 are a more preferred embodiment of the present invention.

The present invention provides examples of particular nucleotide and amino acid mutations at positions corresponding to C24, E26 and C91 of HPV16 E7 and C63 and C106 of HPV16 E6. For example, the present invention provides cysteine 24 to be mutated to glycine (the corresponding mutation in the nucleotide sequence is CTG to CGG). These examples are not limiting. For example, mutations that similarly resulted in disruption of the zinc fingers or Rb binding can be used. For example, mutations at cysteine residues important for zinc finger formation can be changed to any other amino acid. Preferred mutations result in a destabilization of the protein and thus, an increase in the immunogenicity of the protein.

In a specific embodiment of the invention, the polypeptides are fusions of HPV16 E6 and E7 in which the E7 polypeptide has mutations at any amino acid(s) corresponding to positions 24, 26 or 91 of SEQ ID NO: 14 and the E6 polypeptide has either no mutations or has mutations at one or more amino acid(s) corresponding to positions 63 or 106 of SEQ ID NO: 13. In a preferred embodiment of the invention, the polypeptides are fusions of HPV16 E6 and E7 in which the E7 polypeptide has mutations at amino acids corresponding to positions 24 and 26 of SEQ ID NO: 14 and the E6 polypeptide has mutations selected from the group consisting of amino acids corresponding to positions 63 or 106 of SEQ ID NO: 13 or both.

It is understood in the art that the amino acid number is determined by counting

methionine as the first amino acid, even in the case where the first methionine has been deleted in the second of the fusion proteins. For example, the G24 mutation of E6E7TetM (SEQ ID NO: 3) is considered to be at residue 24 of E7 in the polypeptide.

5 In another preferred embodiment of the present invention, the polynucleotides are fusions of HPV16 E6 and E7 polynucleotides in which the E6 polynucleotide has mutations at any of nucleotides 187-189 (which corresponds to nucleotides 290-292 of the HPV16 genome (GenBank accession number K02718)) or 316-318 (which corresponds to nucleotides 419-421 of the HPV16 genome), and the E7 nucleotide sequence has mutations at any of nucleotides 70-72  
10 (which corresponds to nucleotides 631-633 of the HPV16 genome), 76-78 (which corresponds to nucleotides 637-639 of the HPV16 genome) or 271-273 (which corresponds to nucleotides 832-834 of the HPV16 genome). These nucleotide changes result in missense mutations, not nonsense mutations. In other words, these mutations result in a different amino acid being encoded.

15 The present invention provides polypeptides, and immunogenic and pharmaceutical compositions comprising these polypeptides, or comprising nucleotides encoding these polypeptides. As described *infra* these polypeptides and polynucleotides are described in the sequences provided herein. For example, the E6E7TetM polypeptide is described in SEQ ID NO: 3. Those skilled in the art will appreciate that the polypeptide and polynucleotide sequences  
20 of this invention are not limited to the exact sequences disclosed in this application. For example, the E6 and E7 sequences were obtained by performing PCR from the ATCC clone #45113 of HPV16. The E6 and E7 sequences of this ATCC clone vary slightly from those in the GenBank HPV16 sequence, K02718. Thus, those skilled in the art will appreciate that the invention also comprises substantially homologous or substantially similar amino acid and  
25 nucleotide sequences to those disclosed herewith and that the combination of mutations described can occur in the background of any E6 or E7 sequence. For example, these mutations and any number of combinations of these mutations can be in the context of the E6 and E7 sequences disclosed in K02718.

Those skilled in the art will appreciate that this invention can also be drawn to the other  
30 members of the papillomaviral family in addition to HPV16. Other papillomavirus genotypes associated with cancer, in particular, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 have

conserved motifs in the E6 and E7 proteins that are likely central to their oncogenic capacity (see Fields Virology Fourth Edition, 2001, Ch. 65 and 66, 2197-2265, Knipe & Howley Eds., Lippincott Williams and Wilkins). Notably, these E6 and E7 proteins have C-X-X-C zinc finger motifs and, for E7, a putative Rb binding motif L-X-C-X-E. Thus, an embodiment of the present invention comprises E6 and E7 fusion polypeptides and polynucleotides from other members of the papillomavirus family, which have mutations that correspond with the mutations disclosed in the present invention.

Amino acids and nucleotides that correspond to the positions of the particular amino acids and nucleotides disclosed in SEQ ID NOS: 1-12 can be determined by performing a sequence alignment. Examples of such sequence alignments are shown in Figures 2A-E and 3A-C. In these figures, the amino acids of the E6 and E7 polypeptides of HPVs 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 have been aligned, and consensus sequences are presented. The E6 consensus amino acid sequence is represented in SEQ ID NO: 39 and the E7 consensus amino acid sequence is represented in SEQ ID NO: 40.

These alignments demonstrate that each of the mutations tested in the present invention are conserved in each of these HPV viruses and, thus, they too can be mutated in order to knock-out their oncogenic potential, while maintaining their ability to elicit an immune response. Amino acids that are involved in the formation of structures such as zinc fingers and binding motifs are often conserved. One can, for example, identify and mutate the conserved amino acids or nucleotides that correspond to amino acids 24, 26, and 91 of HPV16's E7 and to amino acids 63 or 106 of HPV16's E6 in each of the members of the HPV genotypes. For example, the alignment shown in Figures 2A-E demonstrates that the HPV18 E6 amino acid that corresponds to amino acid 63 of HPV16 E6 (<sup>63</sup>C) is also a cysteine residue and that it is at position 65 of HPV18's E6. Accordingly, <sup>66</sup>C of HPV18's E6, in addition to other residues that correspond to amino acids 24, 26, and 91 of HPV16's E7 and to amino acid 106 of HPV16's E6, can be mutated in the present invention. Similarly, mutations in any HPV E6 polypeptide corresponding to amino acids 65 or 108 of the E6 consensus sequence (SEQ ID NO: 39) or in any HPV E7 polypeptide corresponding to amino acids 25, 27 or 97 of the E7 consensus sequence (SEQ ID NO: 40) can be made in the present invention.

In one embodiment of the present invention, the fusion polypeptides comprise two of these mutations. In a further embodiment, these fusion polypeptides comprise three of these



mutations. In another embodiment, these fusion polypeptides comprise four of these mutations.

In yet another embodiment, these fusion polypeptides comprise all five of these mutations.

**Molecular Biology Definitions.** In accordance with the present invention, there may be employed conventional molecular biology, microbiology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (referred to herein as "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* (B.D. Hames and S.J. Higgins, eds. 1984); *Animal Cell Culture* (R.I. Freshney, ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B.E. Perbal, *A Practical Guide to Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

The polynucleotides herein may be flanked by natural regulatory sequences, or may be associated with heterologous sequences, including promoters, enhancers, response elements, signal sequences, polyadenylation sequences, introns, 5'- and 3'-non-coding regions and the like. The nucleic acids may also be modified by many means known in the art. Non-limiting examples of such modifications include methylation, "caps", substitution of one or more of the naturally occurring nucleotides (i.e., codon optimization of the third or initial "wobble" base), and internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoroamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.). Polynucleotides may contain one or more additional covalently linked moieties, such as proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), intercalators (e.g., acridine, psoralen, etc.), chelators (e.g., metals, radioactive metals, iron, oxidative metals, etc.) and alkylators to name a few. The polynucleotides may be derivatized by formation of a methyl or ethyl phosphotriester or an alkyl phosphoramidite linkage. Furthermore, the polynucleotides herein may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels include radioisotopes, fluorescent molecules, biotin and the like. Other non-limiting examples of modification which may be made are provided, below, in the description of the present invention.

The term "gene" also called a "structural gene" means a DNA sequence that codes for or corresponds to a particular sequence of amino acids which comprise all or part of one or more proteins or enzymes, and may or may not include regulatory DNA sequences, such as promoter sequences, which determine for example the conditions under which the gene is expressed.

- 5 Some genes, which are not structural genes, may be transcribed from DNA to RNA, but are not translated into an amino acid sequence. Other genes may function as regulators of structural genes or as regulators of DNA transcription.

- 10 A "coding sequence" or a sequence "encoding" a polypeptide, protein or enzyme is a nucleotide sequence that, when expressed, results in the production of that polypeptide, protein or enzyme, *i.e.*, the nucleotide sequence encodes an amino acid sequence for that polypeptide, protein or enzyme. Preferably, the coding sequence is an RNA sequence that is translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon near the 5' terminus and a downstream translation stop codon. A coding sequence can include, but is not
- 15 limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

- 20 Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences. "Expression control sequences" are the transcriptional control sequences involved in the initiation of transcription, such as promoters and enhancers.

- 25 A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining this invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. As described above, promoter DNA is a DNA sequence which initiates, regulates, or otherwise
- 30 mediates or controls the expression of the coding DNA.

Amplification of a polynucleotide, as used herein, denotes use of polymerase chain reaction (PCR) to increase the concentration of a particular DNA sequence within a mixture of DNA sequences. For a description of PCR see Saiki et al., Science 1988, 239:487.

The terms "express" and "expression" mean allowing or causing the information in a gene or DNA sequence to become manifest, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an "expression product" such as a protein. The expression product itself, e.g. the resulting protein, may also be said to be "expressed" by the cell. A polynucleotide or polypeptide is expressed recombinantly, for example, when it is expressed or produced in a foreign host cell under the control of a foreign or native promoter, or in a native host cell under the control of a foreign promoter.

The term "transfection" means the introduction of a foreign nucleic acid into a cell. The term "transformation" means the introduction of a "foreign" (*i.e.* extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence, which may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "transformed" and is a "transformant" or a "clone." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or cells of a different genus or species.

The terms "vector", and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression of a polypeptide (e.g. transcription and translation) of the introduced sequence.

Vectors typically comprise the DNA of a transmissible agent, into which foreign DNA is inserted. A common way to insert one segment of DNA into another segment of DNA involves the use of enzymes called restriction enzymes that cleave DNA at specific sites (specific groups

of nucleotides) called restriction sites. Generally, foreign DNA is inserted at one or more restriction sites of the vector DNA, and then is carried by the vector into a host cell along with the transmissible vector DNA. A segment or sequence of DNA having inserted or added DNA, such as an expression vector, can also be called a "DNA construct."

5 A common type of vector is a "plasmid", which generally is a self-contained molecule of double-stranded DNA. A plasmid can readily accept additional (foreign) DNA and which can readily be introduced into a suitable host cell. A plasmid vector often contains coding DNA and promoter DNA and has one or more restriction sites suitable for inserting foreign DNA. Promoter DNA and coding DNA may be from the same gene or from different genes, and may  
10 be from the same or different organisms. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts. Non-limiting examples include pKK plasmids (Clontech), pUC plasmids, pET plasmids (Novagen, Inc., Madison, WI), pRSET or pREP plasmids (Invitrogen, San Diego, CA), or pMAL plasmids (New England Biolabs, Beverly, MA), and many appropriate host cells,  
15 using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. Recombinant cloning vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host, e.g. antibiotic resistance, and one or more expression cassettes. Routine experimentation in biotechnology can be used to determine which cloning vectors are best suited for used with the invention. In general, the choice of  
20 cloning vector depends on the size of the polynucleotide sequence and the host cells to be used.

A "polypeptide" is a chain of chemical building blocks called amino acids that are linked together by chemical bonds called "peptide bonds." The term "protein" refers to polypeptides that contain the amino acid residues encoded by a gene or by a nucleic acid molecule (e.g., an mRNA or a cDNA) transcribed from that gene either directly or indirectly. Optionally, a protein  
25 may lack certain amino acid residues that are encoded by a gene or by an mRNA. A protein or polypeptide, including an enzyme, may be a "native" or "wild-type," meaning that it occurs in nature; or it may be a "mutant," "variant" or "modified," meaning that it has been made, altered, derived, or is in some way different or changed from a native protein or from another mutant.

"Mutation" means any process or mechanism resulting in a mutant protein, enzyme,  
30 polypeptide, polynucleotide, gene, or cell. This includes any mutation in which a protein, enzyme, polynucleotide, or gene sequence is altered, and any detectable change in a cell arising

from such a mutation the altered protein, enzyme, polypeptide or nucleotide is a "mutant," also called a "variant." Typically, a mutation occurs in a polynucleotide or gene sequence, by point mutations (substitutions), deletions, or insertions of single or multiple nucleotide residues. A mutation includes polynucleotide alterations arising within a protein-encoding region of a gene as well as alterations in regions outside of a protein-encoding sequence, such as, but not limited to, regulatory or promoter sequences. A mutation in a gene can be "silent," *i.e.*, not reflected in an amino acid alteration upon expression, leading to a "sequence-conservative" variant of the gene. This generally arises when one amino acid corresponds to more than one codon. Table 1 outlines which amino acids correspond to which codon(s).

Thus, due to the degeneracy of the genetic code, any three-nucleotide codon that encodes a mutated amino acid residue of an E6/E7 fusion polypeptide described herein is within the scope of the invention.

The terms "mutant" and "variant" may also be used to indicate a modified or altered gene, DNA or RNA sequence, enzyme, cell, *etc.*, *i.e.*, any kind of mutant. Such changes also include changes in the promoter, ribosome binding site, *etc.*

The term "variant amino acid sequences" refers to other suitable E6 and E7 fusion polypeptides which can differ from the specifically exemplified E6 and E7 fusion polypeptides by modifications which do not diminish immunogenicity. In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art (Kyte and Doolittle, 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid residue determines the secondary and tertiary structure of the resultant polypeptide, which in turn defines the

interaction of the polypeptide with other molecules, such as enzyme substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydrophobic index and still obtain a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophobic indices are within  $\pm 2$  is preferred, those within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biologically functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. U.S. Patent No. 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the polypeptide.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0  $\pm 1$ ); glutamate (+3.0  $\pm 1$ ); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5  $\pm 1$ ); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred; those within  $\pm 1$  are particularly preferred; and those within  $\pm 0.5$  are even more particularly preferred.

As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take several of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

"Function-conservative variants" are proteins or enzymes in which a given amino acid residue has been changed without altering the overall structural conformation and specified function of the protein or enzyme. This includes but is not limited to, replacement of an amino

acid with one having a polar structural or physical properties, including polar or non-polar character, size, shape and charge (see, e.g., Table 1).

**TABLE 1**

Amino Acids, Corresponding Codons, and Functionality/Property

Amino Acid	SLC	DNA codons	Side Chain Property
Isoleucine	I	ATT, ATC, ATA	Hydrophobic
Leucine	L	CTT, CTC, CTA, CTG, TTA, TTG	Hydrophobic
Valine	V	GTT, GTC, GTA, GTG	Hydrophobic
Phenylalanine	F	TTT, TTC	Aromatic side chain
Methionine	M	ATG	Sulfur group
Cysteine	C	TGT, TGC	Sulfur group
Alanine	A	GCT, GCC, GCA, GCG	Hydrophobic
Glycine	G	GGT, GGC, GGA, GGG	Hydrophobic
Proline	P	CCT, CCC, CCA, CCG	Secondary amine
Threonine	T	ACT, ACC, ACA, ACG	Aliphatic hydroxyl
Serine	S	TCT, TCC, TCA, TCG, AGT, AGC	Aliphatic hydroxyl
Tyrosine	T	TAT, TAC	Aromatic side chain
Tryptophan	W	TGG	Aromatic side chain
Glutamine	Q	CAA, CAG	Amide group
Asparagine	N	AAT, AAC	Amide group

Histidine	H	CAT, CAC	Basic side chain
Glutamic acid	E	GAA, GAG	Acidic side chain
Aspartic Acid	D	GAT, GAC	Acidic side chain
Lysine	K	AAA, AAG	Basic side chain
Arginine	R	CGT, CGC, CGA, CGG, AGA, AGG	Basic side chain
Stop codons	Stop	TAA, TAG, TGA	-

As referred to herein, "sequence similarity" means the extent to which nucleotide or protein sequences are related. The extent of similarity between two sequences can be based on percent sequence identity and/or conservation. Amino acids other than those indicated as conserved may differ in a protein or enzyme so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and can be, for example, at least 70%, even more preferably 80%, and most preferably at least 90%, as determined according to an alignment scheme.

"Sequence identity" herein means the extent to which two nucleotide or amino acid sequences are invariant.

Two DNA sequences are substantially homologous or substantially similar when at least about 80%, and most preferably at least about 90 or 95% of the nucleotides match over the defined length of the DNA sequences, as determined by sequence comparison algorithms, such as BLAST, FASTA, DNA Strider, etc. An example of such a sequence is an allelic or species variant of the specific genes of the invention. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system.

Similarly, two amino acid sequences are substantially homologous or substantially similar when greater than 80% of the amino acids are identical, or greater than about 90% are similar. Preferably, the similar or homologous sequences are identified by sequence alignment.



"Sequence-conservative variants" of a polynucleotide sequence are those in which a change of one or more nucleotides within a given codon results in no alteration in the amino acid encoded by that codon.

"Sequence alignment" means the process of lining up two or more sequences to achieve maximal levels of sequence identity (and, in the case of amino acid sequences, conservation), e.g., for the purpose of assessing the degree of sequence similarity. Numerous methods for aligning sequences and assessing similarity and/or identity are known in the art such as, for example, the Cluster Method, wherein similarity is based on the MEGALIGN algorithm, as well as BLASTN, BLASTP, and FASTA (Lipman and Pearson, 1985; Pearson and Lipman, 1988). When using all of these programs, the preferred settings are those that result in the highest sequence similarity.

The term "heterologous" refers to a combination of elements not naturally occurring. For example, heterologous DNA refers to DNA that is not naturally located in the cell, or in a chromosomal site of the cell. Preferably, heterologous DNA includes a gene foreign to the cell. A heterologous expression regulatory element is a regulatory element operatively associated with a different gene than the one it is operatively associated with in nature.

Modifications, which do not normally alter the primary sequence of the E6 and E7 fusion polypeptides, include *in vivo* or *in vitro* chemical derivatization of polypeptides, e.g., acetylation, methylation, or carboxylation. Also included as variant polypeptides of this invention are these polypeptides modified by glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; or by exposing the polypeptide to enzymes which affect glycosylation, such as mammalian glycosylating or deglycosylating enzymes. Also embraced as variant polypeptides are the above-identified mutagenized sequences, which have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

The term "host cell" means any cell of any organism that is selected, modified, transformed, grown, or used or manipulated in any way, for the production of a substance by the cell, for example the expression by the cell of a gene, a DNA or RNA sequence, a protein or an enzyme.

As used herein, the term "isolated" means that the referenced material is removed from the environment in which it is normally found. Thus, an isolated biological material can be free of cellular components, i.e., components of the cells in which the material is found or produced. In the case of nucleic acid molecules, an isolated nucleic acid includes a PCR product, an isolated mRNA, a cDNA, or a restriction fragment. In another embodiment, an isolated nucleic acid is preferably excised from the chromosome in which it may be found, and more preferably is no longer joined to non-regulatory, non-coding regions, or to other genes, located upstream or downstream of the gene contained by the isolated nucleic acid molecule when found in the chromosome. In yet another embodiment, the isolated nucleic acid lacks one or more introns. Isolated nucleic acid molecules include sequences inserted into plasmids, cosmids, artificial chromosomes, and the like. Thus, in a specific embodiment, a recombinant nucleic acid is an isolated nucleic acid. An isolated protein may be associated with other proteins or nucleic acids, or both, with which it associates in the cell, or with cellular membranes if it is a membrane-associated protein. An isolated organelle, cell, or tissue is removed from the anatomical site in which it is found in an organism. An isolated material may be, but need not be, purified.

The term "purified" as used herein refers to material that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, including native materials from which the material is obtained. For example, a purified protein is preferably substantially free of other proteins or nucleic acids with which it is associated in a cell; a purified nucleic acid molecule is preferably substantially free of proteins or other unrelated nucleic acid molecules with which it can be found within a cell. As used herein, the term "substantially free" is used operationally, in the context of analytical testing of the material. Preferably, purified material substantially free of contaminants is at least 50% pure; more preferably, at least 90% pure, and more preferably still at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, and other methods known in the art.

Methods for purification are well-known in the art. For example, nucleic acids can be purified by precipitation, chromatography (including preparative solid phase chromatography, oligonucleotide hybridization, and triple helix chromatography), ultracentrifugation, and other means. Polypeptides and proteins can be purified by various methods including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, precipitation and salting-out

chromatography, extraction, and countercurrent distribution. For some purposes, it is preferable to produce the polypeptide in a recombinant system in which the protein contains an additional sequence tag that facilitates purification, such as, but not limited to, a polyhistidine sequence, or a sequence that specifically binds to an antibody, such as FLAG and GST. The polypeptide can then be purified from a crude lysate of the host cell by chromatography on an appropriate solid-phase matrix. Alternatively, antibodies produced against the protein or against peptides derived there from can be used as purification reagents. Cells can be purified by various techniques, including centrifugation, matrix separation (e.g., nylon wool separation), panning and other immunoselection techniques, depletion (e.g., complement depletion of contaminating cells), and cell sorting (e.g., fluorescence activated cell sorting [FACS]). Other purification methods are possible. A purified material may contain less than about 50%, preferably less than about 75%, and most preferably less than about 90%, of the cellular components with which it was originally associated. The "substantially pure" indicates the highest degree of purity which can be achieved using conventional purification techniques known in the art.

Polynucleotides are "hybridizable" to each other when at least one strand of one polynucleotide can anneal to another polynucleotide under defined stringency conditions. Stringency of hybridization is determined, e.g., by the temperature at which hybridization and/or washing is performed, and b) the ionic strength and polarity (e.g., formamide) of the hybridization and washing solutions, as well as other parameters. Hybridization requires that the two polynucleotides contain substantially complementary sequences; depending on the stringency of hybridization, however, mismatches may be tolerated. Typically, hybridization of two sequences at high stringency (such as, for example, in an aqueous solution of 0.5×SSC at 65°C) requires that the sequences exhibit some high degree of complementarity over their entire sequence. Conditions of intermediate stringency (such as, for example, an aqueous solution of 2×SSC at 65°C) and low stringency (such as, for example, an aqueous solution of 2×SSC at 55°C), require correspondingly less overall complementarity between the hybridizing sequences. (1×SSC is 0.15 M NaCl, 0.015 M Na citrate.) Polynucleotides that "hybridize" to the polynucleotides herein may be of any length. In one embodiment, such polynucleotides are at least 10, preferably at least 15 and most preferably at least 20 nucleotides long. In another embodiment, polynucleotides that hybridize are of about the same length. In another embodiment, polynucleotides that hybridize include those which anneal under suitable

stringency conditions which encode polypeptides or enzymes having the same function, such as the ability to bind p53 (in the case of E6) or bind Rb (in the case of E7):

The general genetic engineering tools and techniques discussed here, including transformation and expression, the use of host cells, vectors, expression systems, etc., are well known in the art.

As used herein, the term "about" or "approximately" means within 50% of a given value, preferably within 20%, more preferably within 10%, more preferably still within 5%, and most preferably within 1% of a given value. Alternatively, the term "about" or "approximately" means that a value can fall within a scientifically acceptable error range for that type of value, which will depend on how qualitative a measurement can be given the available tools. "About" or "approximately" may define a distribution around a mean value, rather than a single value.

#### **The immunogenic and pharmaceutical compositions of the invention**

The present invention provides immunogenic and pharmaceutical compositions comprising fusion polypeptides and polynucleotides of human papillomavirus E6 and E7. These compositions can be used to treat and/or prevent papillomavirus-induced cancers, such as cervical cancer, and cervical lesions such as CIN. These compositions can also be used to treat lower gastrointestinal tract cancers, such as anal cancer, and other cancers of the reproductive system, such as penile and vulvar cancer.

In a further embodiment, the present invention provides immunogenic and pharmaceutical compositions comprising fusions of polypeptides and fusions of polynucleotides of multiple papillomaviruses. For example, the present invention provides immunogenic and pharmaceutical compositions comprising fusions of E6 and E7 proteins from multiple members of the HPV family such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Examples of HPV E6 polypeptides are shown in Figures 2A-E and the amino acid sequences of E6 from HPV 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are set forth in SEQ ID NOS: 15-26, respectively. The nucleotide sequences encoding these HPV E6 polypeptides are set forth in SEQ ID NOS: 42-53, respectively. Examples of HPV E7 polypeptides are shown in Figures 3A-C and the amino acid sequences of E7 from HPV 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68

are set forth in SEQ ID NOS: 27-38, respectively. The nucleotide sequences encoding these HPV E7 polypeptides are set forth in SEQ ID NOS: 54-65, respectively. These fusions contain mutations in the residues identified by alignment to correspond to the residues mutated in HPV16 described herein or that correspond to the conserved sequences as shown in the E6 and E7 consensus sequences (SEQ ID NOS: 39 and 40, respectively) that interfere with the protein's oncogenic characteristic without interfering with its ability to induce an immune response. In one embodiment of the present invention the immunogenic and pharmaceutical compositions comprise one fusion comprising E6 and E7 polypeptides from different members of the papillomavirus family. For example, a fusion can comprise in a single construct any possible combination of E6 and E7 from HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

The number of E6/E7 polypeptides present in one fusion is limited only by the size limitations of the delivery mechanism or vector in which these compositions are delivered. For example, viruses, constrained by structural limitations, can only package a particular amount of nucleic acid. Those of ordinary skill in the art appreciate the capacity of different viruses to package nucleic acid and appreciate that it is routine to screen for nucleic acids that are of appropriate size for packaging.

Alternatively, the immunogenic and pharmaceutical compositions of the present invention can comprise multiple, different fusions of E6/E7. For example, the immunogenic or pharmaceutical composition can comprise multiple viral particles, each containing different fusions of E6 and E7 sequences from different papillomaviruses.

Immunogenic and pharmaceutical compositions comprising fusions of E6/E7 from multiple papillomaviruses are particularly advantageous because they generate an immune response to multiple E6 and E7 proteins and thus prevent cancers and neoplasias caused by each of these viruses. For example, each of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 has been associated with cervical carcinoma and/or intraepithelial neoplasias (Harrison's Principles of Internal Medicine Fifteenth Edition, 2001, 1119; Braunwald et al., Eds., McGraw-Hill). Thus, a fusion of, for example, HPV16 and 18 E6 and E7 polypeptides would provide prevention and treatment of cancers of two different etiologies. Compositions comprising E6 and E7 polypeptides from multiple, different viruses are a particularly powerful means to treat and/or prevent a wide range of papillomavirus-induced cancers, such as cervical cancer, cervical

lesions such as CIN, or gastrointestinal tract cancers, such as anal cancer, and other cancers of the reproductive system, such as penile and vulvar cancer.

The skilled artisan will appreciate methods of determining the safety of immunogenic compositions. Exemplary methods for determining whether, for example, E6/E7 fusions have decreased or abrogated transforming and immortalization capacity include: determining p53 and/or Rb levels by, for example, Western blot, soft agar assays, transfecting primary keratinocytes or mammary epithelial cells and identifying loss of senescence, and transfecting cells and identifying changes in cell morphology. In addition to these functional assays, biochemical assays can be used. For example, the binding of E6 and E7 to cellular proteins such as p53, E6 TP-1, telomerase and Rb can be measured.

Assessing the levels of p53 and Rb is one method for determining the safety of recombinant viral particles, such as VEE, comprising E6/E7 fusions. The steady state levels of p53 and Rb in primary human MECs were assessed following infection with VRP-expressing wildtype HPV16 E6 and E7, as fusion or individual proteins, E7E6 TetM fusion protein, or GFP as a negative control. VRP infection of MECs revealed grossly reduced levels of p53 and Rb in cultures containing wildtype forms of E6 and E7, respectively; simply fusing E7 to E6 was not sufficient to impair the activity of either protein (Figures 8A and B). In contrast, MECs infected with E7E6 TetM VRPs containing E6 ( $^{63}\text{C}$  and  $^{106}\text{C}$ ) and E7 ( $^{24}\text{C}$  and  $^{26}\text{E}$ ) mutations contained normal levels of p53 and Rb (Figures 8A and B), indicating these four mutations extinguished the primary oncogenic activity of these proteins.

Because eradication of established tumors requires the induction of a strong T cell mediated immune response against the tumor specific viral antigens E6 and E7, Venezuelan equine encephalitis (VEE) replicons were chosen as the immunogenic composition vector. However, any gene or protein delivery method can be used to deliver and package the immunogenic compositions of the present invention. For example, other viral vectors known to those skilled in the art may be used or the nucleotides encoding the mutant fusion polypeptides may be delivered directly by a plasmid.

Recombinant AV vectors, of which VEE is a member, are particularly versatile because they may be launched as naked RNA, naked plasmid DNA or as particles, with the latter being the most effective formulation for use in immunogenic compositions. One distinguishing property of VRPs in contrast to the other AV replicons is their tropism for dendritic cells

(MacDonald, G.H. et al. *Virology* 2000, 74:914-922). VRP-targeted dendritic cells may be highly effective vehicles for conveying antigen into lymph nodes and may be an effective dose-sparing strategy.

5 The immunogenic compositions, particularly the nucleic acids, of the present invention can be delivered via viral vectors, such as lentiviruses, retroviruses, herpes viruses, adenoviruses, adeno-associated viruses, vaccinia virus, baculovirus, alphaviruses and other recombinant viruses with desirable cellular tropism. A wide variety of alphaviruses may be used as viral vectors, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR 67; ATCC VR 1247), Ross River virus (ATCC VR 373; ATCC VR 1246) and Venezuelan equine encephalitis 10 virus (ATCC VR 923; ATCC VR 1250; ATCC VR 1249; ATCC VR 532). Representative examples of such vector systems include those described in U.S. Patent Nos. 5,091,309; 5,217,879; and 5,185,440; and International Patent Publication Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994. Targeting to specific regions of cells is also provided, for example, targeting so that the fusion polypeptide is localized to the cell 15 membrane, as described in U.S. Patent No. 6,228,621.

Thus, a gene encoding an E6/E7 polypeptide fusion can be introduced *in vivo*, *ex vivo*, or *in vitro* using a viral vector or through direct introduction of a nucleic acid, such as a DNA or replicon RNA. Expression in targeted tissues can be effected by targeting the recombinant vector to specific cells, such as with a viral vector or a receptor ligand, or by using a tissue-specific promoter, or both. Targeted gene delivery is described in International Patent 20 Publication WO 95/28494.

Viral vectors commonly used for *in vivo* or *ex vivo* targeting and therapy procedures are DNA-based vectors and retroviral vectors. Methods for constructing and using viral vectors are known in the art (see, e.g., Miller and Rosman, *BioTechniques* 1992, 7:980-990). Preferably, the 25 viral vectors are replication defective, that is, they are unable to replicate autonomously in the target cell. In general, the genomes of the replication defective viral vectors which are used within the scope of the present invention lack at least one region which is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or rendered non-functional by any technique known to a person skilled in the art. These 30 techniques include the total deletion, substitution (by other sequences, in particular by the inserted nucleic acid), partial deletion or addition of one or more bases to an essential (for

replication) region (so as not to cause a frame shift). Such techniques may be performed *in vitro* (on the isolated DNA) or *in situ*, using the techniques of genetic manipulation or by treatment with mutagenic agents. Preferably, the replication defective virus retains the sequences of its genome which are necessary for encapsidating the viral particles.

5           Viral vectors include an attenuated or defective DNA or RNA virus, such as, but not limited to, herpes simplex virus (HSV), Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), VEE, and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. Defective viruses do not generate progeny after introduction into the cell. Use of defective viral vectors allows for administration to cells in a specific, localized area,  
10           without concern that the viral vector will spread and infect other cells. Thus, a particular tissue can be specifically targeted. Examples of particular vectors include, but are not limited to, the replication defective VEE system as described by Pushko et al. (Virology 1997, 239:389-401), and an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al. (J. Clin. Invest. 1992, 90:626-630; see also La Salle et al., Science 1993, 259:988-990), and a  
15           defective adeno-associated virus vector (Samulski et al., J. Virol. 1987, 61:3096-3101; Samulski et al., J. Virol. 1989, 63:3822-3828; Lebkowski et al., Mol. Cell. Biol. 1988, 8:3988-3996).

          Various companies produce viral vectors commercially, including but by no means limited to Avigen, Inc. (Alameda, CA; AAV vectors), Cell Genesys (Foster City, CA; retroviral, adenoviral, AAV vectors, and lentiviral vectors), Clontech (retroviral and baculoviral vectors),  
20           Genovo, Inc. (Sharon Hill, PA; adenoviral and AAV vectors), Genvec (adenoviral vectors), IntroGene (Leiden, Netherlands; adenoviral vectors), Molecular Medicine (retroviral, adenoviral, and AAV vectors), Norgen (adenoviral vectors), Oxford BioMedica (Oxford, United Kingdom; lentiviral vectors), Transgene (Strasbourg, France; adenoviral, vaccinia, retroviral, and lentiviral vectors), AlphaVax (alphaviral vectors such as VEE vectors) and Invitrogen (Carlsbad,  
25           California).

          In another embodiment, the vector can be introduced *in vivo* by lipofection, as naked DNA, or with other transfection facilitating agents (peptides, polymers, bupivacaine etc.). Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. U.S.A. 1987, 84:7413-7417; Felgner  
30           and Ringold, Science 1989, 337:387-388; Mackey et al., Proc. Natl. Acad. Sci. U.S.A. 1988, 85:8027-8031; Ulmer et al., Science 1993, 259:1745-1748). Useful lipid compounds and



compositions for transfer of nucleic acids are described in International Patent Publications WO 95/18863 and WO 96/17823, and in U.S. Patent No. 5,459,127. Lipids may be chemically coupled to other molecules for the purpose of targeting (see, Mackey et al., Proc. Natl. Acad. Sci. U.S.A. 1988, 85:8027-8031). Targeted peptides, e.g., hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically. Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (e.g., International Patent Publication WO 95/21931), peptides derived from DNA binding proteins (e.g., International Patent Publication WO 96/25508), or a cationic polymer (e.g., International Patent Publication WO 95/21931).

It is also possible to introduce the vector *in vivo* as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., electroporation, microinjection, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun (for example, the Helios gene gun system (Bio-Rad; Hercules, CA) can be used for epidermal gene delivery), or use of a DNA vector transporter (see, e.g., Wu et al., J. Biol. Chem. 1992, 267:963-967; Wu and Wu, J. Biol. Chem. 1988, 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990; Williams et al., Proc. Natl. Acad. Sci. U.S.A. 1991, 88:2726-2730). Receptor-mediated DNA delivery approaches can also be used (Curiel et al., Hum. Gene Ther. 1992, 3:147-154; Wu and Wu, J. Biol. Chem. 1987, 262:4429-4432). U.S. Patent Nos. 5,580,859 and 5,589,466 disclose delivery of exogenous DNA sequences, free of transfection facilitating agents, in a mammal. Alternatively, the DNA is formulated in compositions with transfection facilitating agents, which facilitate immunization, such as bupivacaine and other local anesthetics (U.S. Patent No. 6,127,170). Recently, a relatively low voltage, high efficiency *in vivo* DNA transfer technique, termed electrotransfer, has been described (Mir et al., C.P. Acad. Sci. 1998, 321:893; WO 99/01157; WO 99/01158; WO 99/01175).

The term "immunogenic composition", as used herein, broadly refers to any compositions that may be administered to elicit an immunogenic response in the recipient. An immunogenic composition generally comprises an immunologically effective dose of an immunogen (e.g., an antigen of an infectious agent) and a pharmaceutically acceptable carrier and, optionally, an adjuvant. The pharmaceutically acceptable carrier may be sterile water or sterile isotonic saline, as well as any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with

administration to number 1. The appropriate carrier will be evident to those skilled in the art and will depend in large part upon the route of administration.

5 An immunogenic composition may be administered to an organism, e.g., by inhalation or insufflation (either through the mouth or the nose), or by oral, buccal, vaginal, rectal or parenteral administration (e.g., by subcutaneous, intradermal, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intraperitoneal or intravenous injection and the like). An immunogenic composition may also be administered by particle-mediated transfer (e.g., using a “particle gun”). See for example, Gainer et al., J. Neurooncol 2000, 47:23-30; Koide et al., Jpn J. Pharmacol 2000, 83:167-174; Kuriyama et al., Gene Ther. 2000, 7:1132-1136; and Yamauchi et al., J. Exp. Zool. 2000, 287:285-293. Such particle transfer methods are particularly preferred for DNA or vector immunogenic compositions, e.g., using a “gene gun.” The appropriate route of administration is selected depending on the nature of the immunogenic composition used, and an evaluation of the age, weight, sex and general health of the patient and the antigens present in the immunogenic composition, and similar factors by an attending physician.

15 An immunogenic composition may comprise, for example, a suspension of an attenuated or killed infectious agent (e.g., a microorganism such as a bacterium or a virus, a parasite or other pathogen, etc.) that causes an infectious disease. Alternatively an immunogenic composition of the invention may be a polypeptide immunogenic compositions or a DNA immunogenic composition. The term “polypeptide immunogenic composition” refers to an immunogenic composition comprising an immunogenic polypeptide, for example a polypeptide derived from an infectious agent which may be an antigen, and therefore activates an immune response in an organism. The term “DNA immunogenic composition” is used herein to refer to immunogenic compositions delivered by means of a recombinant vector. An alternative term used herein is “vector immunogenic composition” (since some potential vectors, for example alphaviruses, are RNA viruses and since in some instances non-viral RNA instead of DNA may be delivered to cells).

30 The term “immunologically effective dose” refers to that amount of a compound or compositions that is sufficient to result in a desired activity. Thus, as used to describe an immunogenic composition, an immunologically effective dose refers to the amount of a compound or compositions (e.g., an antigen) that is sufficient to produce an effective immune response. In general, selection of the appropriate immunologically effective amount or dosage

for the immunogenic compositions of the present invention will also be based upon the particular immunogenic composition employed, as well as the physical condition of the subject, most especially including the general health and weight of the immunized subject. Such selection and upward or downward adjustment of the effective dose is within the skill of the art. The amount of active component required to induce an immune response without significant adverse side effects varies depending upon the composition employed.

For recombinant, preferably replication-defective, viruses containing the DNA encoding the mutant E6/E7 fusion polypeptides of this invention, the immunologically effective amount is an amount of recombinant virus that is effective in a route of administration to transfect the desired cells of the subject and provide sufficient levels of expression of the selected gene to provide the desired effect. The levels of immunity can be monitored to determine the need, if any, for boosters.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve, e.g., as a tissue depot that slowly releases the antigen, and also as a lymphoid system activator that enhances the immune response (see, Hood et al., Immunology, Second Ed., 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Such adjuvants also include, among others, MPL™ (3-O-deacylated monophosphoryl lipid A; Corixa, Hamilton, MT), which is described in U.S. Patent No. 4,912,094, which is hereby incorporated by reference. Also suitable for use as adjuvants are aminoalkyl glucosamine phosphate compounds (AGP), or derivatives or analogs thereof, which are available from Corixa (Hamilton, MT), and which are described in United States Patent No. 6,113,918, which is hereby incorporated by reference. One such AGP is 2-[(R)-3-Tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-O-phosphono-3-O-[(R)-3-tetradecanoyoxytetradecanoyl]-2-[(R)-3-tetradecanoyoxytetradecanoylamino]-b-D-glucopyranoside, which is also known as 529 (formerly known as RC529). This 529 adjuvant is formulated as an aqueous form or as a stable emulsion.

Other adjuvants include mineral oil and water emulsions, aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, etc., Amphigen, Avridine, L121/squalene, D-lactide-poly lactide/glycoside, muramyl dipeptide, killed Bordetella, saponins, such as Quil A or Stimulon™ QS-21 (Antigenics, Framingham, MA.), described in U.S. Patent No. 5,057,540, which is hereby incorporated by reference, and particles generated therefrom such as ISCOMS

(immunostimulating complexes), *Mycobacterium tuberculosis*, bacterial lipopolysaccharides, synthetic polynucleotides such as oligonucleotides containing a CpG motif (U.S. Patent No. 6,207,646, which is hereby incorporated by reference), cholera toxin (either in a wild-type or mutant form, e.g., wherein the glutamic acid at amino acid position 29 is replaced by another amino acid, preferably a histidine, in accordance with International Patent Publication No. WO 00/18434, incorporated herein by reference), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129; see, e.g., International Patent Publication Nos. WO 93/13302 and WO 92/19265, incorporated herein by reference. Various cytokines and lymphokines are suitable for use as adjuvants. One such adjuvant is granulocyte-macrophage colony stimulating factor (GM-CSF), which has a nucleotide sequence as described in U.S. Patent No. 5,078,996, which is hereby incorporated by reference. A plasmid containing GM-CSF cDNA has been transformed into *E. coli* and has been deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, under Accession Number 39900. The cytokine Interleukin-12 (IL-12) is another adjuvant that is described in U.S. Patent No. 5,723,127, which is hereby incorporated by reference. Other cytokines or lymphokines have been shown to have immune modulating activity, including, but not limited to, the interleukins 1-alpha, 1-beta, 2, 4, 5, 6, 7, 8, 10, 13, 14, 15, 16, 17 and 18, the interferons-alpha, beta and gamma, granulocyte colony stimulating factor, and the tumor necrosis factors alpha and beta, and are suitable for use as adjuvants.

Other suitable adjuvants include, but are not limited to: surface active substances (e.g., hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyldioctadecylammonium bromide), methoxyhexadecylglycerol, and pluronic polyols; polyamines, e.g., pyran, dextran sulfate, poly IC, carbopol; peptides, e.g., muramyl dipeptide, dimethylglycine, tuftsin; oil emulsions; and mineral gels, e.g., aluminum phosphate, etc. and immune stimulating complexes. The immunogen may also be incorporated into liposomes, or conjugated to polysaccharides, lipopolysaccharides and/or other polymers for use in an immunogenic composition.

Exemplary adjuvants include, but are not limited to, incomplete Freund's adjuvant, surface active substances (for example, lysolecithin), pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions. Exemplary adjuvants also include potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. In addition, immunostimulatory proteins, such as chemokines, or nucleic acid sequences encoding for

chemokines, may be provided as an adjuvant to increase the immune response to an immunogenic composition.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction (for example, gastric upset, dizziness and the like) when administered to an individual. Preferably, and particularly where an immunogenic composition is used in humans, the term "pharmaceutically acceptable" may mean approved by a regulatory agency (for example, the U.S. Food and Drug Agency) or listed in a generally recognized pharmacopeia for use in animals (for example, the U.S. Pharmacopeia).

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a compound is administered. Sterile water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Exemplary suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures, for example in cell culture assays or using experimental animals to determine the LD50 and the ED50. The parameters LD50 and ED50 are well known in the art, and refer to the doses of a compound that are lethal to 50% of a population and therapeutically effective in 50% of a population, respectively. The dose ratio between toxic and therapeutic effects is referred to as the therapeutic index and may be expressed as the ratio: LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used. However, in such instances it is particularly preferable to use delivery systems that specifically target such compounds to the site of affected tissue so as to minimize potential damage to other cells, tissues or organs and to reduce side effects.

Data obtained from cell culture assay or animal studies may be used to formulate a range of dosages for use in humans. The dosage of compounds used in therapeutic methods of the present invention preferably lie within a range of circulating concentrations that includes the ED50 concentration but with little or no toxicity (e.g., below the LD50 concentration). The particular dosage used in any application may vary within this range, depending upon factors such as the particular dosage form employed, the route of administration utilized, the conditions of the individual (e.g., patient), and so forth.

Non-human animals include, without limitation, laboratory animals such as mice, rats, rabbits, hamsters, guinea pigs, etc.; domestic animals such as dogs and cats; farm animals such as sheep, goats, pigs, horses, and cows; and non-human primates.

5 A therapeutically effective dose may be initially estimated from cell culture assays and formulated in animal models to achieve a circulating concentration range that includes the IC<sub>50</sub>. The IC<sub>50</sub> concentration of a compound is the concentration that achieves a half-maximal inhibition of symptoms (e.g., as determined from the cell culture assays). Appropriate dosages for use in a particular individual, for example in human patients, may then be more accurately determined using such information.

10 The term "treat" means to attempt to elicit an anti tumor response against cells of the tumor, i.e., the cancer. An anti-tumor response includes, but is not limited to, increased time of survival, inhibition of tumor metastasis, inhibition of tumor growth, tumor regression, and development of a delayed-type hypersensitivity (DTH) response to unmodified tumor cells.

15 Measures of compounds in plasma may be routinely measured in an individual such as a patient by techniques such as high performance liquid chromatography (HPLC) or gas chromatography.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

20 Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by the routes described above.

25 For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may

be presented as a dry product for constitution with water or other suitable vehicle before use.

Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by

implantation (for example subcutaneously or intramuscularly) or by intramuscular injection.

Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

5           The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

10           The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them.

## EXAMPLES

15           The present invention is described by way of the following examples. However, the use of these or other examples anywhere in the specification is illustrative only and in no way limits the scope and meaning of the invention or any exemplified term. Likewise, the invention is not limited to any preferred embodiment described herein. Indeed, many modifications and variations of the invention may be apparent to those skilled in the art upon reading this  
20           specification and can be made without departing from its spirit and scope.

### **Example 1. DESIGN AND GENERATION OF HPV16 IMMUNOGENIC COMPOSITION CONSTRUCTS.**

#### **Materials and Methods**

25           **Generation of VEE-replicon constructs.** The HPV16 E6 and E7 genes were obtained from pHPV-16 (ATCC, #45113) by PCR methods (Horton et al., Gene 1989, 77:61-8), and fused in two different orientations to generate open reading frames (ORFs) of 744 base pairs (bp) encoding 248 amino acids for all constructs. The methionine start codon of each downstream ORF was removed to eliminate any possibility of internal initiation. Specific nucleotides of the



(Stratagene; La Jolla, CA). The wild-type (wt) and mutated (mut) fused genes were sub-cloned into the vector pVR200 (AlphaVax; Durham, NC), a plasmid derived from the cDNA of a highly attenuated, non-neurotrophic mutant (V3014) of the Trinidad Donkey strain of Venezuelan equine encephalitis (VEE) (Grieder, F.B. et al., Virology 1995, 206:994-1006). The pVR200 plasmid is described in Pushko et al., (Virology 1997, 239:389-401, in particular see p. 390-391 "Cell lines and plasmids" and p. 393 Figure 1b). Briefly, this plasmid has the T7 promoter followed by VEE's non-structural genes, the subgenomic promoter 26S, the cloning site for the gene(s) of interest (in the present invention, the E6/E7 fusions), and a *NotI* linearization site.

Replication-incompetent VEE replicon particles (VRPs) were prepared by the split helper method and titrated as described (Pushko et al., Virology 1997, 239:389-401). Briefly, the pVR200 plasmid (with the fusions of interest cloned in) was cotransfected into cells along with: (1) a capsid-encoding helper construct, and (2) a glycoprotein-encoding helper construct. Neither of these helper constructs had packaging sequences and therefore were not incorporated into VRPs. Thus, the resulting VRPs were replication defective. The potency of each VRP preparation, expressed as infectious units/milliliter (IU/ml), was defined by the number of E7-positive particles as determined by titration on baby hamster kidney (BHK)-21 cells. Titers of all VRP preparations exceeded  $10^9$  IU per electroporation; an effective dose of  $3 \times 10^5$  IU per immunization was used throughout these studies and as previously described (Velders M.P. et al., Cancer Res 2001, 61:7861-7867).

**Western blots and Immunofluorescence.** BHK-21 cells were infected with the indicated VRPs. Twenty-four hours post-infection the cells were either harvested in SDS sample buffer for Western blot analysis or fixed with methanol-acetone for immunofluorescence. To detect protein expression by Western blot, the proteins in the cell lysate were separated by SDS-PAGE, transferred onto a Polyvinylidene (PVD) membrane and analyzed with the Western Breeze detection system (Invitrogen; Carlsbad, CA) using an anti-HPV16 E7 monoclonal antibody (Zymed; San Francisco, CA). For immunofluorescence analysis, the fixed cells were incubated with a primary anti-E7 antibody followed by FITC-labeled goat anti-mouse secondary antibody (# 554001, Pharmingen; San Diego, CA). The cell nuclei were stained using Viaprobe (#555815, Pharmingen).

## Results

In order to design effective and safe immunogenic compositions against HPV16-induced cervical cancer, antigenic diversity was increased by expressing both E6 and E7 tumor specific antigens. The inclusion of full-length E6 and E7 genes in a VRP immunogenic composition is desirable for maximizing the likelihood of expressing all possible epitopes for stimulating CD8<sup>+</sup> and CD4<sup>+</sup> T cells in HLA Class I and Class II -diverse human populations. Fusions of the E6 and E7 ORFs were accomplished in two different orientations by PCR and up to five amino acids were mutagenized (Figures 1A and B) to obtain expression constructs for producing particular E6/E7 fusion polypeptides.

A single amino acid substitution at position <sup>63</sup>C has been shown to destroy several HPV16 E6 functions: p53 degradation, E6TP-1 degradation, activation of telomerase, and, consequently, immortalization of primary epithelial cells (Gao, Q. et al., J Virol 2001, 75:4459-4466). HPV16 E6 containing a single point mutation at <sup>106</sup>C neither binds nor facilitates degradation of p53 and is incapable of immortalizing human MECs, a phenotype dependent upon p53 degradation (Dalal et al., J Virol 1996, 70:683-688). The 98 amino acid HPV16 E7 protein binds Rb through an L-X-C-X-E motif; mutations at positions <sup>24</sup>C and <sup>26</sup>E of this motif destroy Rb binding and degradation (Munger, K, et al., Oncogene 2001, 20:7888-7898). In addition to these two point mutations in E7, a third amino acid, <sup>91</sup>C, was mutated to destroy the single zinc finger in E7.

A first fusion protein, referred to here as E6E7wt comprises the amino acid sequence of a wild-type E6 polypeptide sequence (SEQ ID NO: 13) at the amino terminus and the amino acid sequence of a wild-type E7 polypeptide sequence (SEQ ID NO: 14) at the carboxy terminus. A representative amino acid sequence for such a fusion polypeptide is provided at SEQ ID NO: 1. An exemplary nucleotide sequence encoding such a wild-type E6E7 fusion polypeptide is set forth in SEQ ID NO: 2.

A second fusion polypeptide, referred to as E6E7TetM, was also prepared. Like the E6E7wt fusion polypeptide, E6E7TetM comprises an E6 polypeptide sequence at the amino terminus and an E7 polypeptide sequence at the carboxy terminus. However, the E6 polypeptide sequence of this construct contains glycine amino acids at residues 63 and 106 of the E6 polypeptide rather than the cysteine amino acids found in the wild-type E6 amino acid sequence (SEQ ID NO: 13). In addition, the E7 polypeptide sequence of this construct contains glycine

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amino acids at residues 24 and 26 of the E7 polypeptide rather than cysteine (24) and  
glutamate (26) amino acids found in the wild-type E7 amino acid sequence (SEQ ID NO: 14). A  
representative amino acid sequence for such a fusion polypeptide is provided at SEQ ID NO: 3.  
An exemplary nucleotide sequence encoding such an E6E7TetM fusion polypeptide is set forth  
5 in SEQ ID NO: 4.

10 A third fusion polypeptide, referred to as E6E7PentM, was also prepared. Like the  
E6E7TetM fusion polypeptide, E6E7PentM comprises an E6 polypeptide sequence, which  
comprises glycine amino acids at residues 63 and 106, at its amino terminus and an E7  
polypeptide sequence, which comprises glycine amino acids at residues 24 and 26, at its carboxy  
terminus. However, the E7 polypeptide sequence of this fusion protein also contains a glycine  
amino acid at residue 91 of the E7 polypeptide sequence, rather than the cysteine amino acid  
found in the wild-type E7 amino acid sequence (SEQ ID NO: 14). A representative amino acid  
sequence for such a fusion polypeptide is provided at SEQ ID NO: 5. An exemplary nucleotide  
sequence encoding such an E6E7PentM fusion polypeptide is set forth in SEQ ID NO: 6.

15 A fourth fusion polypeptide, referred to as E7E6wt, comprises the amino acid sequence  
of a wild-type E7 polypeptide (SEQ ID NO: 14) sequence at the amino terminus and the amino  
acid sequence of a wild-type E6 polypeptide (SEQ ID NO: 13) sequence at the carboxy terminus.  
A representative amino acid sequence for such a fusion polypeptide is provided at SEQ ID NO:  
7. An exemplary nucleotide sequence encoding such an E7E6wt fusion polypeptide is set forth  
20 in SEQ ID NO: 8.

A fifth fusion polypeptide, referred to as E7E6TetM, was also prepared. Like the  
E7E6wt fusion polypeptide, E7E6TetM comprises an E7 polypeptide sequence at the amino  
terminus and an E6 polypeptide sequence at the carboxy terminus. However, the E7 polypeptide  
of this construct contains a glycine residue at amino acids 24 and 26 of the E7 polypeptide, rather  
25 than the cysteine (24) and glutamate (26) amino acids found in the wild-type E7 amino acid  
sequence (SEQ ID NO: 14). In addition, the E6 polypeptide of this construct contains a glycine  
residue at amino acids 63 and 106 of the E6 polypeptide, rather than the cysteine amino acid  
found in the wild-type E6 amino acid sequence (SEQ ID NO: 13). A representative amino acid  
sequence for such a fusion polypeptide is provided at SEQ ID NO: 9. An exemplary nucleotide  
30 sequence encoding such an E7E6TetM fusion polypeptide is set forth in SEQ ID NO: 10.

A sixth fusion polypeptide, referred to as E7E6PentM, was also prepared. Like E7E6TetM, E7E6PentM comprises an E7 polypeptide, which comprises glycine amino acids at residues 24 and 26 of the E7 polypeptide, at its amino terminus and an E6 polypeptide, which comprises glycine amino acids at residues 63 and 106 of the E6 polypeptide, at its carboxy terminus. However, the E7 polypeptide sequence of this construct also contains a glycine amino acid at residue 91 of the E7 polypeptide, rather than the cysteine amino acid found in the wild-type E7 amino acid sequence (SEQ ID NO: 14). A representative amino acid sequence for such a fusion polypeptide is provided at SEQ ID NO: 11. An exemplary nucleotide sequence encoding such an E7E6TetM fusion polypeptide is set forth in SEQ ID NO: 12.

The mutations in E6 and E7 were designed to 1) disrupt three zinc fingers, therefore destabilizing the protein and accelerating degradation (and thus increase immunogenicity) ( $^{63}\text{C}$  and  $^{106}\text{C}$  of E6;  $^{91}\text{C}$  of E7 (Dalal et al., J Virol 1996, 70:683-8; Shi et al., J Virol 1999, 73:7877-81)); 2) perturb E6-induced degradation of p53 ( $^{106}\text{C}$ ; (Dalal et al., J Virol 1996, 70:683-8)) and E6-TP1-binding (Gao et al., J Virol 2001, 75:4459-66); and 3) disrupt Rb binding and degradation by E7 ( $^{24}\text{C}$  and  $^{26}\text{E}$ ; (Edmonds and Vousden, J Virol 1989, 63:2650-6)). These mutations were carefully chosen to lie outside known HLA epitopes, with the exception of  $^{91}\text{C}$ . Although  $^{91}\text{C}$  is known to be part of an HLA A2 epitope that spans amino acids 86-93 of E7 (Ressing et al., J Immunol 1995, 154:5934-43; Ressing et al., Cancer Res 1996, 56:582-8; Evans et al., Cancer Res 1997, 57:2943-50), it was decided to mutate this amino acid to achieve maximum immunogenic composition safety, since this mutation disrupts a zinc finger of E7 known to be required for its immortalizing activity (Jewers et al., J. Virol 1992, 66:1329-1335). Since this mutation it is not located in the P2 amino acid anchor region of peptides with known HLA-A\*0201 binding properties (Rammensee et al., Annu Rev Immunol 1993, 11:213-44), it is possible that C91G mutated polypeptides may retain their ability to bind the HLA-A\*0201 molecule. Also, it is possible that the C91 mutation might affect the cleavage of another HLA epitope spanning amino acids 82-90 of E7. The fusion constructs containing those five amino acid mutations were named PentM.

Although mutations at each of these sites of HPV16 E6 and E7 had been previously disclosed individually, it was not known what combinations of these mutations, if any, would maintain their immunogenicity.

In order to develop a therapeutic cervical cancer immunogen composition that elicits a robust cell-mediated response against the viral oncogene products E6 and E7, these fusions were cloned into the Venezuelan equine encephalitis (VEE) virus replicon-based system. The advantages of recombinant VEE immunogenic compositions include high levels of heterologous gene expression, dendritic-cell tropism (thereby targeting expression to lymphoid tissues, an important site for inducing immunity), induction of apoptosis and robust cellular and humoral immune responses and efficient repeated immunization, since there is no widespread existing immunity to VEE in humans. In addition, alphaviruses such as VEE replicate the RNA of interest in the cell cytosol and are cytopathic, thereby significantly reducing the risk of integration of E6 and E7 into the cellular genome.

To assess the expression of these fused constructs, BHK cells were infected with the recombinant VRPs and subsequently analyzed by Western blotting and immunofluorescence. Western blot analysis revealed that the E6E7 fusion proteins migrated generally at a molecular weight of about 30 kDa on SDS-PAGE. Although the expression level of the wildtype and mutated constructs was comparable, their intracellular localization was dramatically different. Immunofluorescence staining of these fusion proteins revealed a punctate staining pattern overlapping the nuclei for both wildtype E6 and E7 VRPs, while a more diffuse, perinuclear staining was observed for all TetM and PentM VRPs (data not shown). Such a diffuse, perinuclear localization is suggestive of protein aggregation/misfolding. Such misfolding is suggestive of protein instability, further supporting that these fusions have an increased capacity to elicit CTL responses.

## **Example 2: CELLULAR IMMUNE RESPONSES INDUCED BY DIFFERENT IMMUNOGENIC COMPOSITION CONSTRUCTS.**

### **Materials and Methods**

**Mice and cell lines.** Specific pathogen-free 6-12 week old female C57BL/6 mice were obtained from Taconic Farms (Germantown, NY). Mice were housed in the Wyeth and Loyola University (Chicago) animal facilities under filtertop conditions, with water and food *ad libitum*. Specific pathogen-free 6-12 week old female HLA-A\*0201 mice were purchased from Jackson Laboratories (Bar Harbor, ME). MC57G and EL4 cells were used for cytotoxicity assays. BHK-

21 cells were used for E RNA expression, VEE replicon particle (VRP) packaging and titration (potency assays). Tumor challenge studies were performed using the E6E7-positive tumor lines C3 (Feltkamp, M.C.W. et al., Eur J Immun 1993, 23:2242-2249), TC-1 (Lin, K.Y., et al., Cancer Res 1996, 56:21-26), and HLF16. All cell lines (except HLF16, C3 and TC-1 cells) were obtained from American Type Culture Collection (ATCC; Manassas, VA).

**Cytotoxicity (CTL) assay.** C57BL/6 mice were immunized subcutaneously with  $3 \times 10^5$  infectious units of VRPs. Cytotoxicity assays were performed 4 weeks after immunizing mice with a single dose of  $3 \times 10^5$  of the indicated VRP administered in the rear footpads. Single-cell splenocyte suspensions were restimulated (20:1) with mitomycin-C-treated MC57G cells infected with recombinant modified Vaccinia Virus Ankara (MVA) vectors encoding E7 or E6 (E7-MVA or E6-MVA) at a multiplicity of infection (MOI) of 5. CTL activity was measured 5 days later. E7- or E6- MVA-infected MC57G cells and HPV16 E7<sub>49-57</sub> H-2D<sup>b</sup>-restricted peptide (RAHYNIVTF (SEQ ID NO: 41, which corresponds to amino acids 49-57 of SEQ ID NO: 14); Lin et al., Cancer Res 1996, 56, 21-6)-pulsed EL-4 cells (ATCC) served as targets. MC57G cells were infected for 1 h with either E7-MVA or E6-MVA at a MOI of 5. EL-4 cells ( $1 \times 10^7$ ) were incubated with peptide (20  $\mu$ g/ml) for 1 h. Target cells were then labeled 3 h later with Europium (Eu<sup>+3</sup>; Sigma Chemical Co., St. Louis, MO) by electroporation. Effector and target cells were incubated at the indicated ratios for 3 h, after which supernatants were harvested and mixed with Enhancer solution (Wallac; Turku, Finland). Eu<sup>+3</sup> release was quantitated by time-resolved fluorescence using a 1234 Delfia fluorometer (Wallac). The percentage of specific lysis was calculated as (Experimental-spontaneous release/ Maximal-spontaneous release) X 100. The percentage spontaneous releases ranged from 5 to 10%.

## Results

To characterize immune responses induced by the different immunogenic composition constructs, C57BL/6 mice were immunized subcutaneously in the rear footpads with  $3 \times 10^5$  infectious units of the wt and PentM VRP constructs. One month after immunization, CTL-mediated lysis was measured by Europium release assay using MC57G targets infected with MVA encoding E6 or E7. Figure 4A reveals that CTL from mice immunized with either wildtype or PentM VRPs killed E7 expressing targets. Identical results were found with E7<sub>49-57</sub> peptide pulsed EL-4 targets (data not shown). CTL mediated lysis was also evident against E6 targets from mice immunized with all forms of VRPs, although lysis was substantially reduced in

recipients of mutant (Figure 4B). These results for E7 and E6 specific lysis were reproduced in two additional experiments and were also observed using TetM VRPs immunized mice (data not shown).

These results show that immunization with VRPs encoding mutant and wildtype fusion proteins generated similar CTL responses to the immunodominant E7<sub>49-57</sub> epitope which is important in tumor rejection. While CTL responses were clearly detectable against E6 targets from animals immunized with VRPs expressing wildtype genes, diminished CTL responses were observed in mice receiving a mutant form of E6, suggesting that <sup>63</sup>C and/or <sup>106</sup>C is an important component of an H-2<sup>b</sup>-restricted epitope.

### 10 **Example 3: TUMOR PROTECTION AND THERAPEUTIC EFFICACY OF IMMUNOGENIC COMPOSITION CONSTRUCTS IN C3 AND TC1 TUMOR MODELS.**

#### **Materials and Methods**

15 **Tumor protection and therapeutic experiments.** Groups of 14 C57BL/6 mice were anesthetized by intraperitoneal injection of 10 mg/kg xylazine (Sigma, St. Louis, MO) and 100 mg/kg ketamine (Abbott Laboratories, Chicago, IL) and immunized by injection of 3x10<sup>5</sup> VRPs into the hind footpads on days -21 and -7. Negative control mice received 3 x 10<sup>5</sup> IU of green fluorescent protein (GFP) VRP. One week later, half of the mice in each group were challenged by subcutaneous flank injections with 5x10<sup>5</sup> C3 cells (Feltkamp et al., Eur J Immunol 1995, 25:2638-42) and half were challenged with 5x10<sup>4</sup> TC-1 cells (Lin et al., Cancer Res 1996, 56:21-6). Tumor growth was monitored every three days. For C3 therapeutic experiments, mice were first challenged with 5 x 10<sup>5</sup> C3 tumor cells in the flank followed 7, 14, and 21 days later with the indicated VRP at 3 x 10<sup>5</sup> dose per immunization. For the human lung fibroblast (HLF) therapeutic experiment, HLA-A\*0201 transgenic mice were challenged with 2x 10<sup>6</sup> HLF16 cells by subcutaneous injection into the left flank on day 0. At 5, 10 and 15 days following challenge, mice were anesthetized as described above and immunized by injection of 3x 10<sup>5</sup> VRPs into the hind footpads. Tumor growth was monitored every 5 days.

See also: Mice and Cell lines (above).

## Results

VRPs encoding wildtype E7E6 and E7E6PentM fusion proteins were compared for prophylactic antitumor efficacy *in vivo*. Mice in all groups were immunized at days 0 and 21 with  $3 \times 10^5$  of the indicated VRP and subsequently challenged with C3 or TC-1 tumor cells. All mice receiving GFP-VRP as a negative control developed tumors within about 7 days post-tumor challenge (Figures 5A and B). In contrast, all mice receiving either E7E6 wildtype or PentM were protected from tumor challenge regardless of whether they received C3 ( $5 \times 10^5$ ) (Figure 5A) or TC-1 ( $5 \times 10^4$ ) (Figure 5B) tumor cells. These data suggest that protection was not limited to a specific murine tumor challenge model and that protection was critically dependent upon VRP-encoded E6 and E7 gene products.

As a more stringent measure of antitumor efficacy, in the next experiment mice were challenged with C3 tumor cells prior to immunization with E7E6 wildtype, PentM, or TetM VRPs at days 7, 14, and 21. Figure 6 shows that 85%-100% of mice receiving any of the mutant or wildtype E6 and E7 fusion-protein-expressing VRPs rejected C3 tumors in contrast to 0%-12% of negative control mice. As shown previously by Velders et al. (Cancer Res 2001, 61:7861-7) and in Figure 6, a VRP immunogenic composition expressing E7 alone promoted rejection in only 65%-75% of mice.

In conclusion, the inclusion of the gene encoding E6 as a fusion partner with E7 reproducibly enhanced therapeutic antitumor efficacy (85%-100%, Figure 6) compared to E7 alone (67%-75%, Figure 6) regardless of whether E6 was wild-type or mutated. Therefore, the mutations in E6, when expressed as a fusion with E7, did not result in a diminished antitumor effect in these tumor models. However, the results illustrate that optimal CTL responses to epitopes containing mutant amino acids may not be elicited in some individuals immunized with mutant VRP. In humans this may not be a cause for concern given the diversity of HLA Class I and Class II alleles compared to the limited number of H-2 alleles expressed by C57BL/6 mice.

### **Example 4: THERAPEUTIC EFFICACY OF IMMUNOGENIC COMPOSITION CONSTRUCTS IN THE HLF16 TUMOR MODEL.**

Although the results of Examples 2 and 3 show encouraging results, H-2b restricted T cell recognition of HPV antigens in C57BL/6 mice provides limited predictive value for HLA



restricted anti-tumor responses. Therefore, the demonstration that the same immunogenic compositions can induce HLA-A\*0201 restricted responses against HPV16 E6 and E7 in HLA-A\*0201 transgenic mice is particularly important (Ressing et al., J Immunol 1995, 154:5934-5943).

5 CD8<sup>+</sup> T cells from HLA-A\*0201 transgenic mice have been shown to recognize the same HLA-A\*0201 restricted antigens as those recognized by HLA-A\*0201 restricted human CTLs (Engelhard et al., J Immunol 1991, 146:1226-1232; Shirai et al., J Immunol 1995, 154:2733-2742). The use of HLA transgenic mice could therefore overcome the limitations of H-2 restricted tumor rejection. However, no HPV tumor model has been available to test HLA-A\*0201 restricted anti-tumor response. Here, the first HPV16 tumor model for HLA-A\*0201 transgenic mice is presented (also described in Eiben GL et al., Cancer Research, Oct. 15 2002, 62, No. 20). The immunogenicity of the E6/E7 fusions was tested in this model.

15 This tumor model was developed by transfecting fibroblasts from HLA-A 0201 transgenic C57BL/6 mice with HPV16 E6 and E7 and Ras V12, generating a cell line (HLF16) that is tumorigenic in HLA-A 0201 mice. The dominant H-2Db epitope was removed from the E7 gene to ensure that the anti-tumor responses would be independent of the 49-57 epitope and would be likely mediated through the HLA-A\*0201.

20 The TetM set of constructs were used in the HLF tumor model. These constructs contained only four mutations: C63G and C106G in E6; C24G and E26G in E7. The mutation C91G in E7 (present in the PentM constructs) was eliminated because it is known to be part of HLA-A\*0201 epitope that spans amino acids 86-93 of E7 (Ressing et al., J Immunol 1995, 154:5934-43; Ressing et al., Cancer Res 1996, 56:582-8; Evans et al., Cancer Res 1997, 57:2943-50). By using a class II-binding algorithm ([www.imtech.res.in/raghava/propred](http://www.imtech.res.in/raghava/propred)), it was also determined that there is a predicted epitope promiscuous for several class II haplotypes between amino acids 87 and 95 of E7. Since class II-mediated immune responses were found to be necessary for the therapeutic efficacy of the VRP immunogenic composition in the C3 tumor model, the hypothesis was that C91G mutation present in the PentM immunogenic composition constructs could disrupt human class II epitopes.

**Construction and characterization of the HLF16 tumor model.** The HLF16 tumor cell line was derived from heart lung tissue dissected from HLA-A2 Dd transgenic C57BL/6 mice. After several weeks in culture, adherent fibroblasts were transformed with a pIRES bi-cistronic vector containing E6/E7 and activated H-ras while conferring geneticin resistance. The only known H-2b Class I restricted epitope from the HPV16 E749-57 gene product (Velders et al., Cancer Res 1997, 61:7861-7867) was removed to ensure that the tumor would not present this immunodominant HPV16 E7 epitope. Transfectants were selected on G418 and clonally expanded. Individual clones were then tested for HLA-A\*0201 expression by FACS analysis. Clones that showed the highest HLA-A\*0201 expression were subsequently tested for their ability to form colonies in soft agar. Heart lung fibroblast clone 16 (HLF16) showed anchorage independent growth in soft agar and was chosen for further studies. E7 expression was evident in the cytoplasm and nucleus of HLF16 after immunofluorescence staining with an anti-E7 monoclonal antibody. To determine if the HLF16 line would in fact form tumors in mice, HLA-A\*0201 transgenic mice were injected with different concentrations of tumor cells and monitored for 35 days. All mice developed tumors but only those challenged with the highest dose,  $2 \times 10^6$  HLF16 cells, maintained a tumor over the time course. The HLF16 tumor arose at approximately day 5 and continued to grow progressively until it became approximately 12x12x12 mm by day 35 at which point the mice were sacrificed. Construction and characteristics of the HLF16 tumor cell line is also described in Eiben GL et al. (Cancer Research, Oct. 15 2002, 62, No. 20).

**Soft Agar Assay.** Anchorage-independent growth capability was determined by assessing the colony formation efficiency of cells suspended in soft agar. Transformed and control cells ( $0.5 \times 10^6$ ,  $0.25 \times 10^6$  and  $0.1 \times 10^6$ ) were seeded in 5 ml of 0.3% overlay agar and added to 10 mm plates coated with 20 ml of 0.6% underlay agar. Plates were allowed to dry and were incubated at 37° C. Colonies were counted 3 weeks after plating.

**See also: Mice and Cell lines, Tumor Protection and Therapeutic Experiments, and Western blots and Immunofluorescence (above).**

## Results

The TetM constructs were checked for expression by Western blot and immunofluorescence of BHK-infected cells. Both E6E7 and E7E6 TetM proteins migrated at about 30 kDa on SDS-PAGE and had a diffuse peri-nuclear localization very similarly to the PentM constructs.

Both PentM and TetM immunogenic composition constructs were analyzed for their tumor therapeutic efficacy in the HLF tumor model (Figure 7). The transgenic mice were challenged with  $2 \times 10^6$  HLF16 cells subcutaneously and at days 5, 10 and 15 after challenge, the mice were immunized with either GFP VRPs, TetM VRPs or PentM VRPs. Complete tumor rejection followed immunization with E7E6 TetM VRPs, while E7E6 PentM VRPs and E6E7 PentM VRPs induced tumor regression of 90% of the mice, except E6E7 TetM (Figure 7). These results demonstrate that therapeutic immunization with several of the VRPs tested here resulted in a high degree of antitumor efficacy independent of contributions by T cells specific for E7<sub>49-57</sub>. Furthermore, these results demonstrate that fusions in which E6 is carboxy terminal to E7 (E7E6 fusions) provide greater immune protection than their counterparts in which E6 is at the amino terminus (E6E7 fusions).

From the collective therapeutic efficacy data (Figures 6 and 7) across C3 and HLF16 tumor models, we can conclude that VRPs encoding mutant forms of HPV16 E6 and E7, without any auxiliary proteins, cytokines, or adjuvants, are highly effective at eradicating established murine tumors.

### **Example 5: MUTANT E6 AND E7 EXPRESSING VRPS DO NOT INDUCE DEGRADATION OF P53 AND RB.**

The steady state levels of p53 and Rb in primary human MECs was assessed following infection with VRPs expressing wildtype HPV16 E6 and E7, as fusion or individual proteins, E7E6 TetM fusion protein, or GFP as a negative control.

## **Materials and Methods**

**Detection of p53 and Rb.** Primary human mammary epithelial cells (MEC, Clonetics, San Diego, CA) were infected with VRPs encoding wildtype or mutant forms of E6 and E7 at

MOI = 10 and total cellular proteins harvested 16-20 hours later. To enhance p53 detection, cells were treated with 1.0 nM actinomycin D (Sigma, St. Louis, MO). Twenty-five micrograms of total protein were loaded per lane, electrophoresed by SDS-PAGE, and blotted to PVDF membranes. Western blots were probed using anti-p53 antibody (FL-393, Santa Cruz Biotech, Santa Cruz, CA) or anti-Rb antibody (Catalog # 554136, BD Pharmingen, San Diego, CA). Tubulin levels were monitored as loading controls by probing with an anti-tubulin antibody (H-235, Santa Cruz Biotech, Santa Cruz, CA).

### Results

It was determined whether a mutant form of E6 and E7 fusion protein, when expressed in the context of VRP infection, would functionally inactivate p53 and Rb in comparison to wildtype versions of these proteins. The E7E6 TetM was selected because it demonstrated high antitumor efficacy (Figures 6 and 7) and contained a minimal number of mutations.

Primary human mammary epithelial cells (MEC) were infected with VRPs encoding HPV16 E6 alone, E7 alone, E7E6 wild-type, or E7E6 TetM. Approximately twenty hours post-infection with a MOI = 10 of each of these VRPs, cell lysates containing equivalent amounts of total cellular protein were electrophoresed by SDS-PAGE, transferred to PVDF membranes, and probed with antibodies specific for p53 (Figure 8A), Rb (Figure 8B), and tubulin as a loading control. The results of these Western blots are shown in Figures 8A and 8B and are representative of two independent experiments.

MEC infected with VRPs encoding E6 alone or E7E6 wildtype fusion protein contained undetectable levels of p53 in contrast to MEC infected with E7E6 TetM which contained p53 levels comparable to negative control GFP-VRP infected samples (Figure 8A). MEC infected with VRPs encoding E7 alone or E7E6 wildtype fusion protein contained undetectable levels of Rb in contrast to MEC infected with E7E6 TetM VRPs or GFP-VRPs (Figure 8B). The presence of E7-containing fusion protein in the E7E6 wildtype and E7E6 TetM VRP infected samples was verified by probing those lanes with an anti-E7 monoclonal antibody (Figures 8A and B). The results show that p53 and Rb levels are grossly diminished in primary MEC expressing wildtype versions of E6 and E7 in the context of a VRP infection but are normal in MEC expressing E7E6 TetM following VRP infection.

in summary, infection of MEC revealed grossly reduced levels of p53 and Rb in

5 cultures containing wildtype forms of E6 and E7, respectively; simply fusing E7 to E6 was not sufficient to impair the activity of either protein (Figures 8A and B). In contrast, MEC infected with E7E6 TetM VRPs containing E6 ( $^{63}\text{C}$  and  $^{106}\text{C}$ ) and E7 ( $^{24}\text{C}$  and  $^{26}\text{E}$ ) mutations contained normal levels of p53 and Rb (Figures 8A and B), indicating these four mutations extinguished the primary oncogenic activity of these proteins. An assessment of the immortalization potential of E7E6 TetM VRPs revealed that MEC died following infection, which is an expected consequence of expression of the AV nonstructural proteins expressed by replicons (Griffith et al., Annu. Rev. Microbiol. 1997, 51:565-592) and further supports the safety of this vector.

10 \* \* \* \* \*

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

15 Numerous references, including patents, patent applications and various publications are cited and discussed in the description of this invention. The citation and/or discussion of such references is provided merely to clarify the description of the present invention and is not an admission that any such reference is "prior art" to the invention described herein. All references cited and/or discussed in this specification are incorporated herein by reference in their entirety  
20 and to the same extent as if each reference was individually incorporated by reference.

1                   1.     A polypeptide comprising human papillomavirus E6 and E7 polypeptides,  
2     wherein the E7 polypeptide has mutations at any one or more of the amino acids  
3     corresponding to amino acids 24, 26 or 91 of SEQ ID NO: 14 and the E6 polypeptide has no  
4     mutations or has mutations at any one or more of the amino acids corresponding to amino  
5     acids 63 or 106 of SEQ ID NO: 13.

1                   2.     The polypeptide of claim 1 wherein the mutated amino acids are mutated  
2     to glycine.

1                   3.     The polypeptide of claim 1 wherein the E7 polypeptide precedes the E6  
2     polypeptide.

1                   4.     The polypeptide of claim 2 wherein the E7 polypeptide precedes the E6  
2     polypeptide.

1                   5.     An isolated nucleic acid encoding the polypeptide of claim 1.

1                   6.     The nucleic acid of claim 5 wherein the nucleotide sequence of E7  
2     precedes the nucleotide sequence of E6.

1                   7.     An expression vector comprising the nucleic acid sequence of claim 5  
2     under the control of an expression control sequence.

1                   8.     A host cell comprising the nucleic acid of claim 5.

1                   9.     A host cell which expresses the polypeptide of claim 1.

1                   10.    A host cell comprising the expression vector of claim 7.

1                   11.    An immunogenic composition comprising:

2                   (a)    the polypeptide of claim 1; and

3                   (b)    a pharmaceutically acceptable carrier.

12. The immunogenic composition of claim 11 further comprising adjuvant.

13. An immunogenic composition comprising the nucleic acid of claim 5.

14. A recombinant virus comprising the nucleic acid of claim 5.

15. The recombinant virus of claim 14, wherein the virus is a modified Venezuelan equine encephalitis virus.

16. A method for producing an immune response in an individual, which method comprises administering to the individual the immunogenic composition of claim 11 in an amount sufficient to produce the immune response.

17. A method of treating cervical cancer, which method comprises administering to a patient diagnosed with cervical cancer the immunogenic composition according to claim 11 in an amount sufficient to produce a protective immune response.

18. A method of preventing cervical cancer, which method comprises administering to an individual the immunogenic composition of claim 11 in an amount sufficient to produce a protective immune response.

19. A method of preventing cervical cancer, which method comprises administering to an individual the expression vector of claim 7 in an amount sufficient to produce a protective immune response.

20. A method of treating cervical cancer, which method comprises administering to a patient diagnosed with cervical cancer the expression vector of claim 7 in an amount sufficient to produce a protective immune response.

21. The isolated polypeptide of claim 1 wherein the E7 polypeptide has mutations in at least two of amino acids corresponding to amino acids 24, 26 and 91 of SEQ ID NO: 14 and the E6 polypeptide has one or more mutations at amino acids corresponding to amino acids 63 and 106 of SEQ ID NO: 13.

22. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9, or SEQ ID NO: 11

1           23.    An isolated nucleic acid encoding a polypeptide comprising the amino  
2 acid sequence set forth in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 9 or SEQ ID NO:  
3 11.

1           24.    The isolated nucleic acid of claim 23 having the nucleotide sequence as set  
2 forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 10 or SEQ ID NO: 12.

1           25.    An expression vector comprising the nucleic acid sequence of claim 23  
2 under the control of an expression control sequence.

1           26.    An expression vector comprising the nucleic acid sequence of claim 24  
2 under the control of an expression control sequence.



Figure 1A

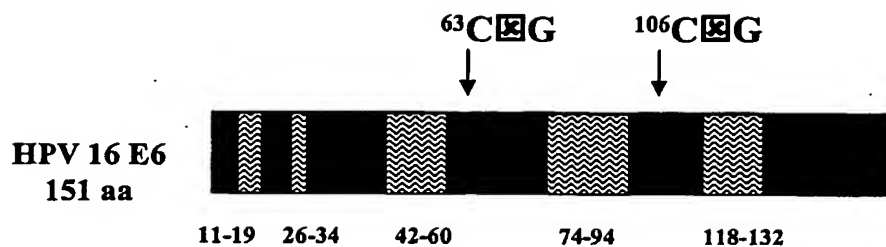
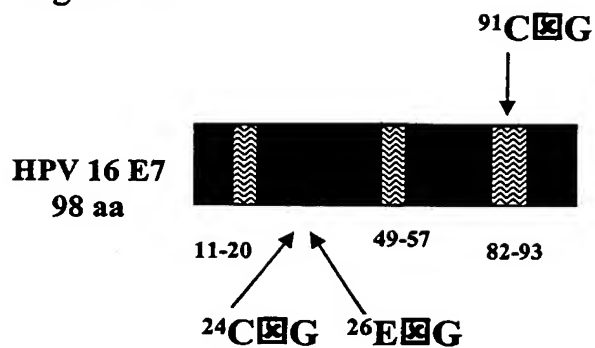


Figure 1B



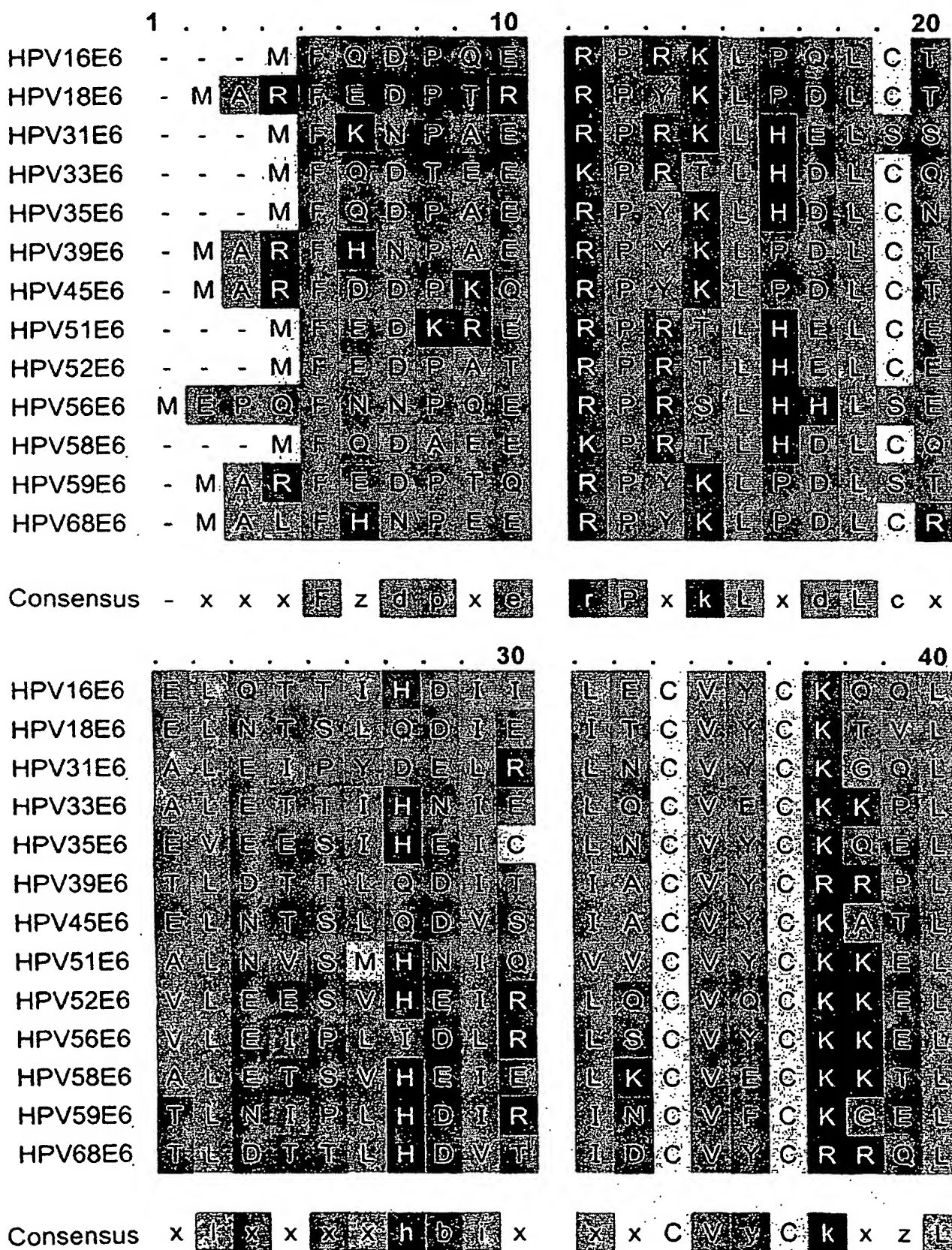


Figure 2A

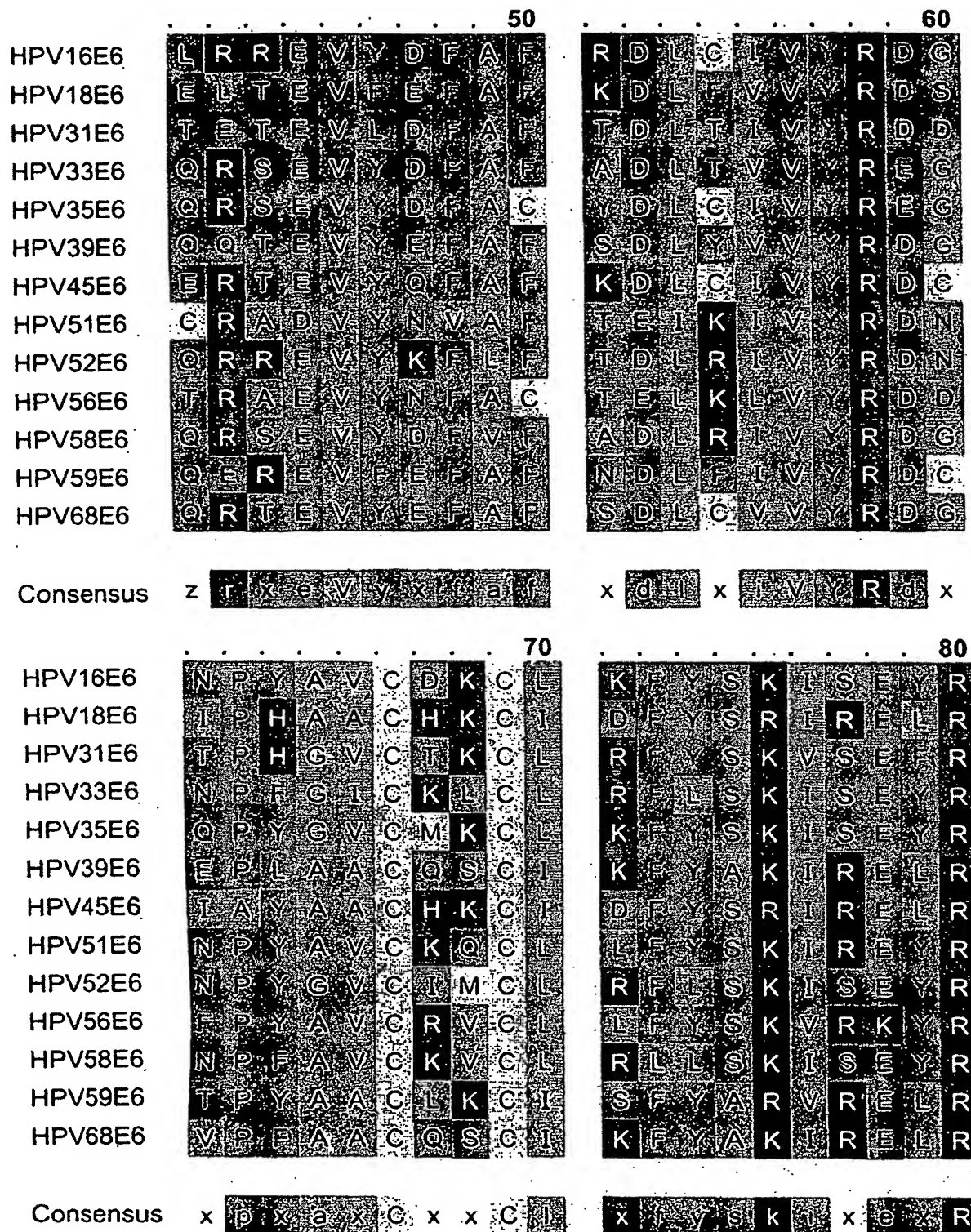


Figure 2B

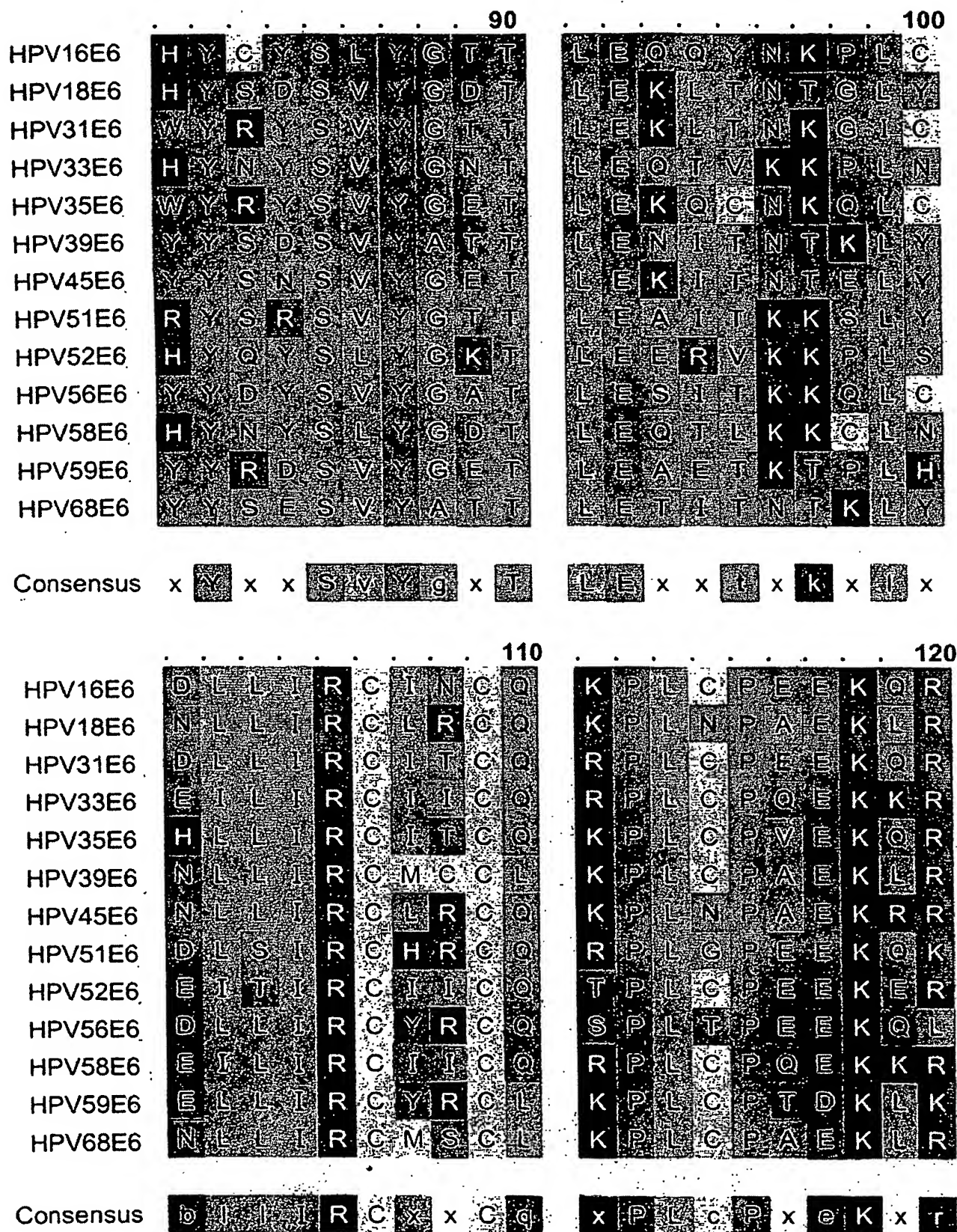


Figure 2C



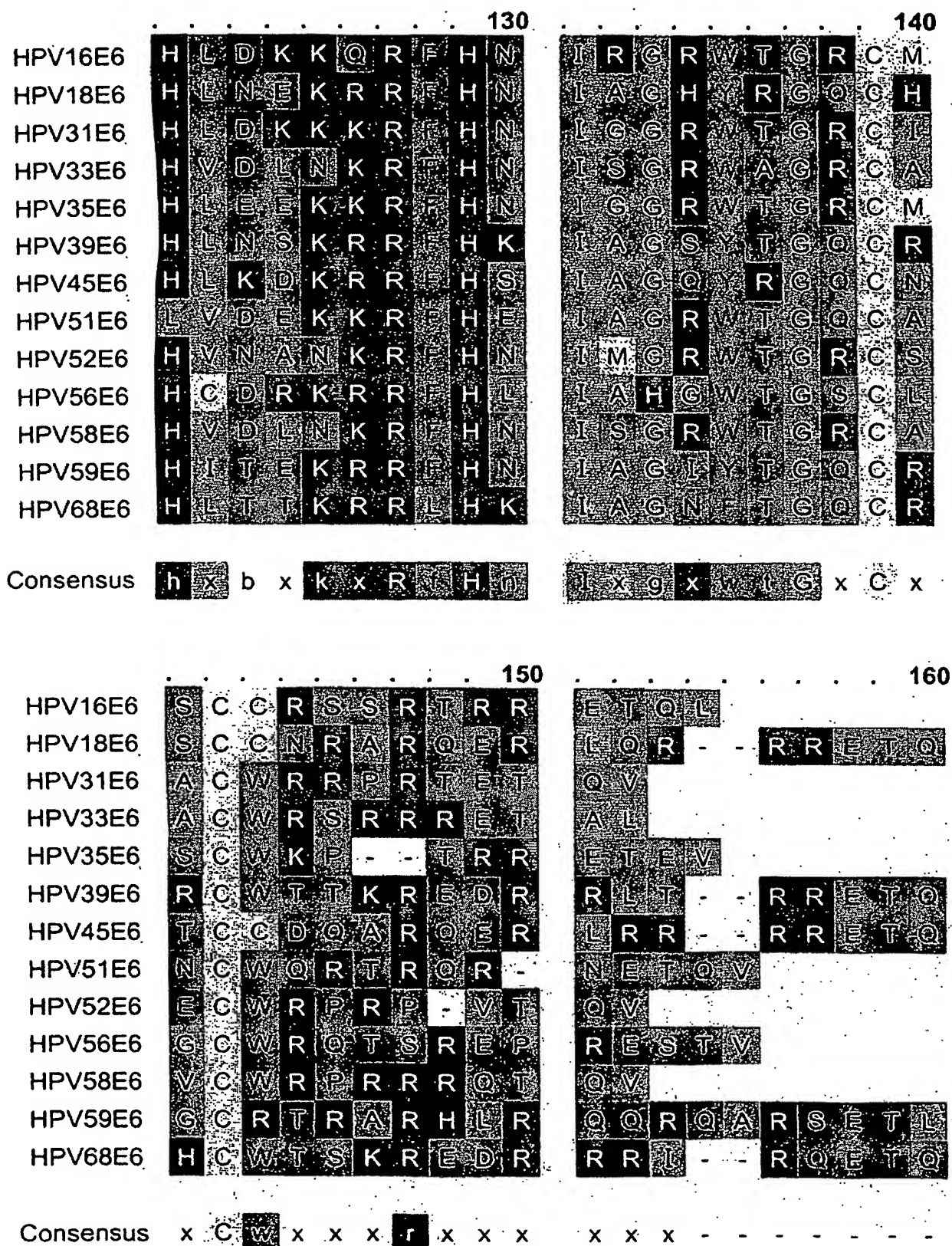
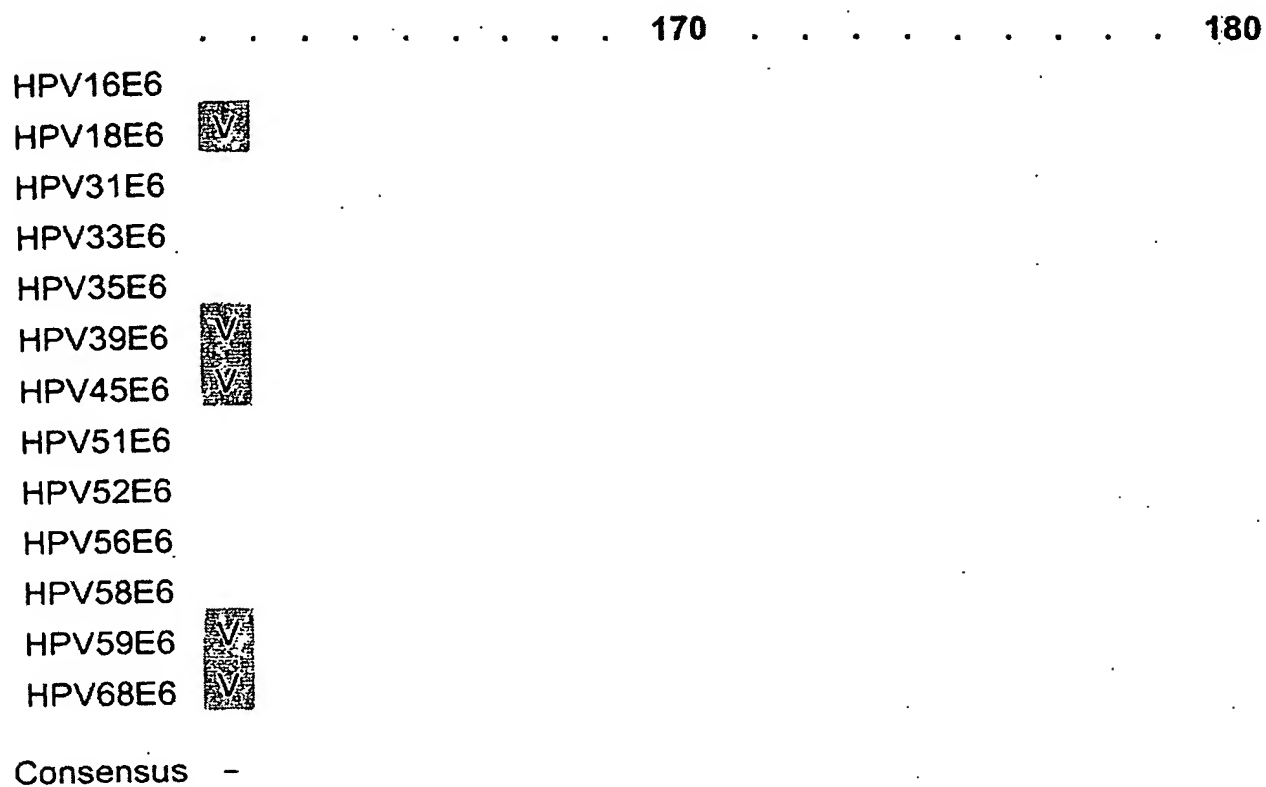


Figure 2D

**Figure 2E**

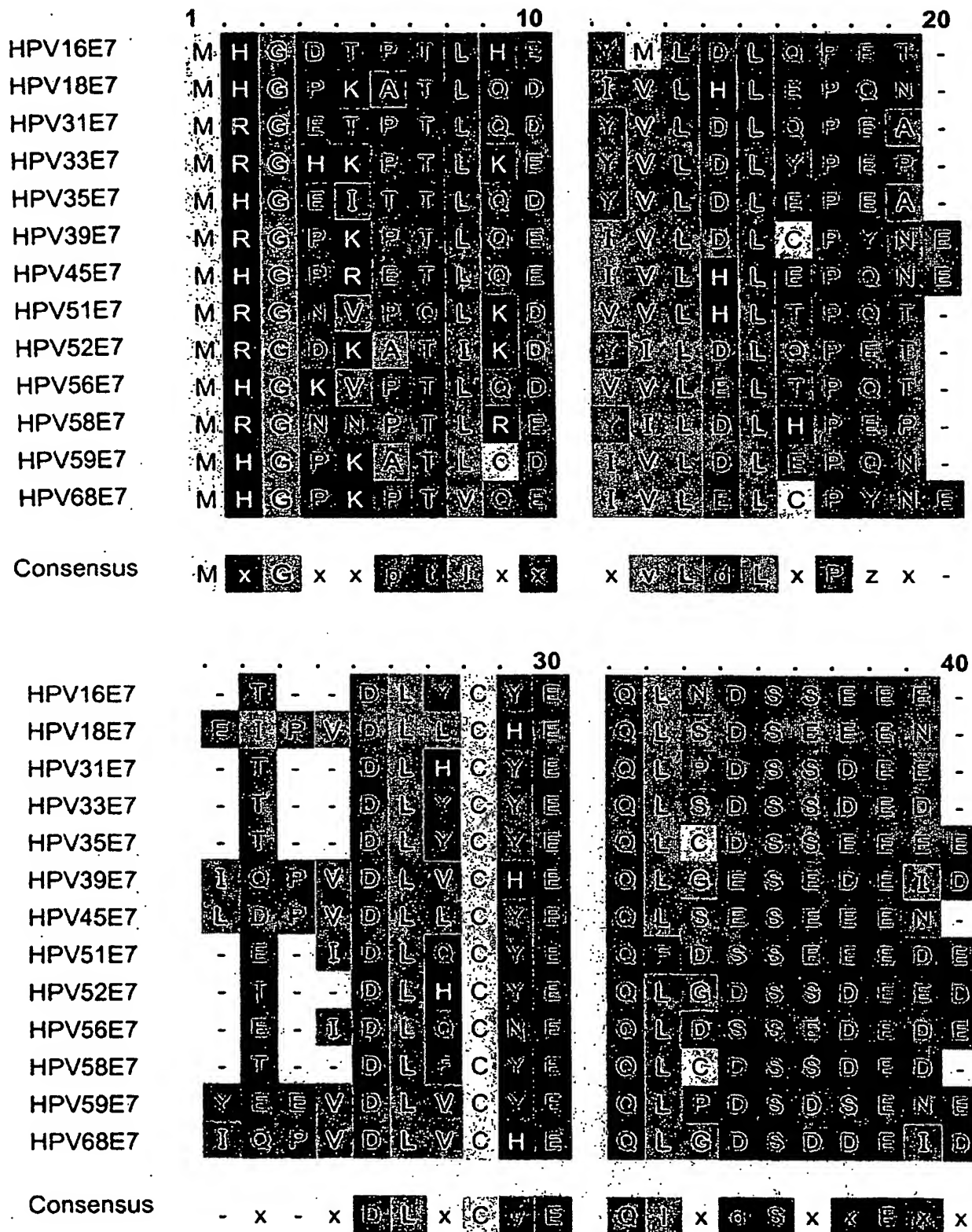


Figure 3A

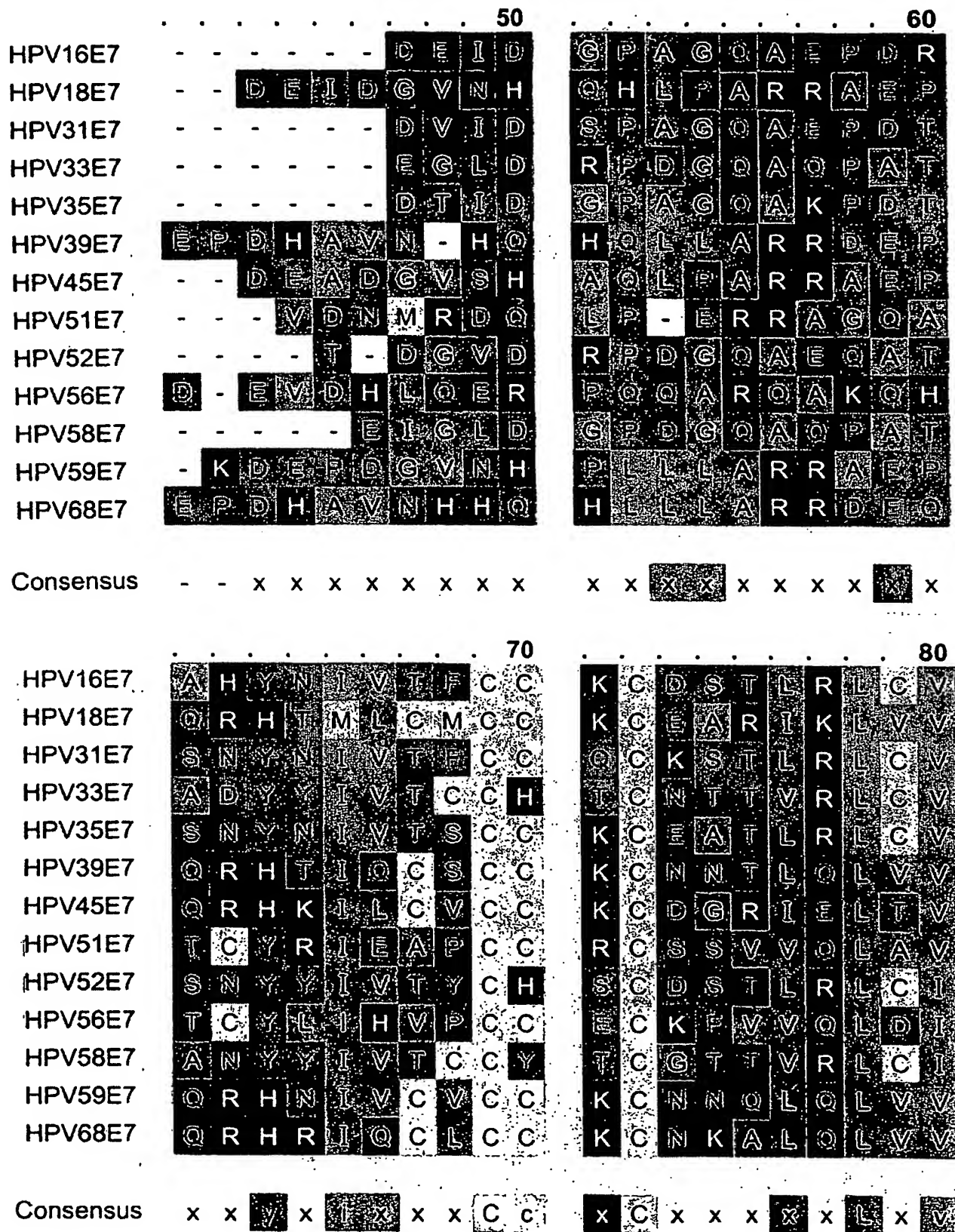


Figure 3B



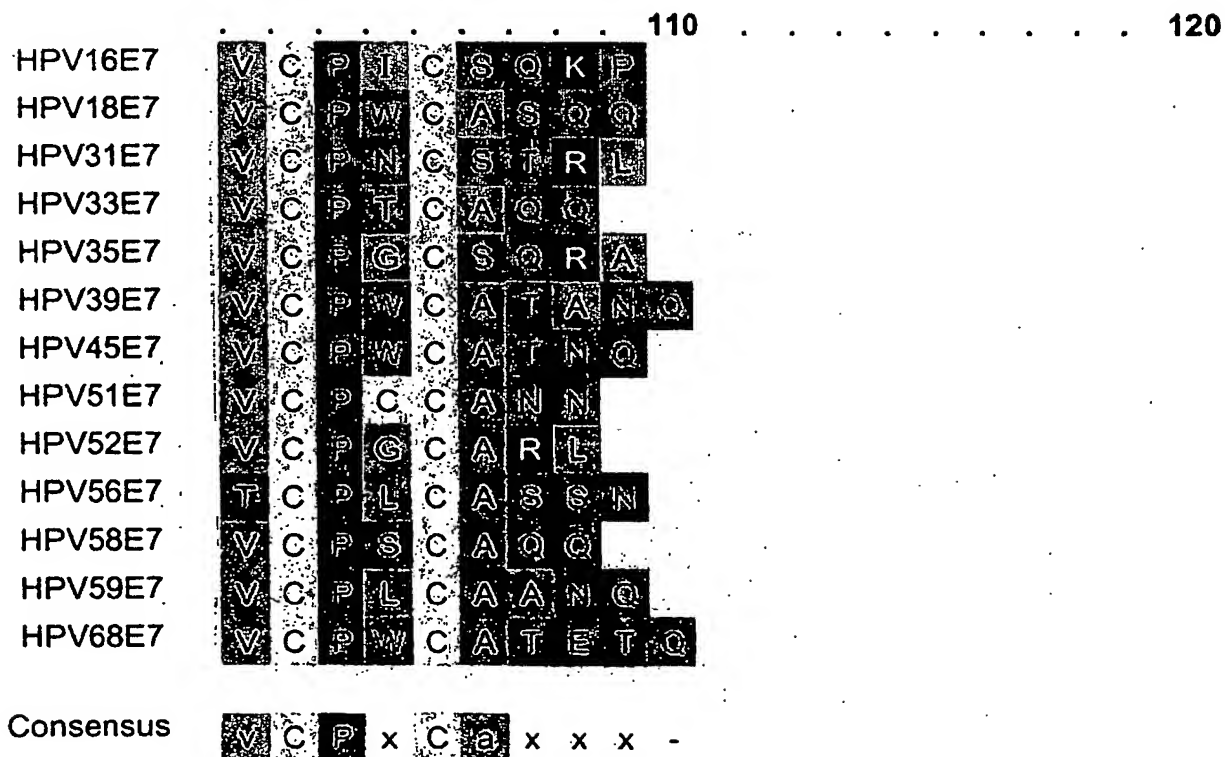
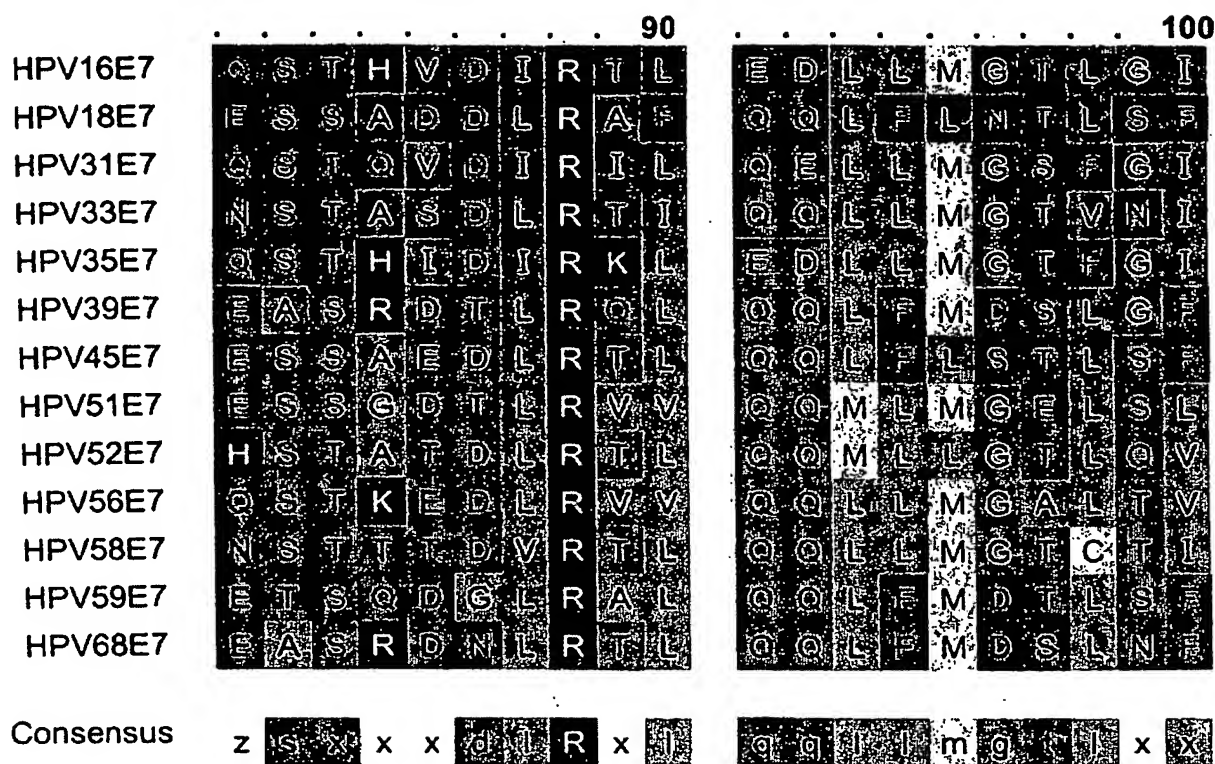


Figure 3C

Figure 4

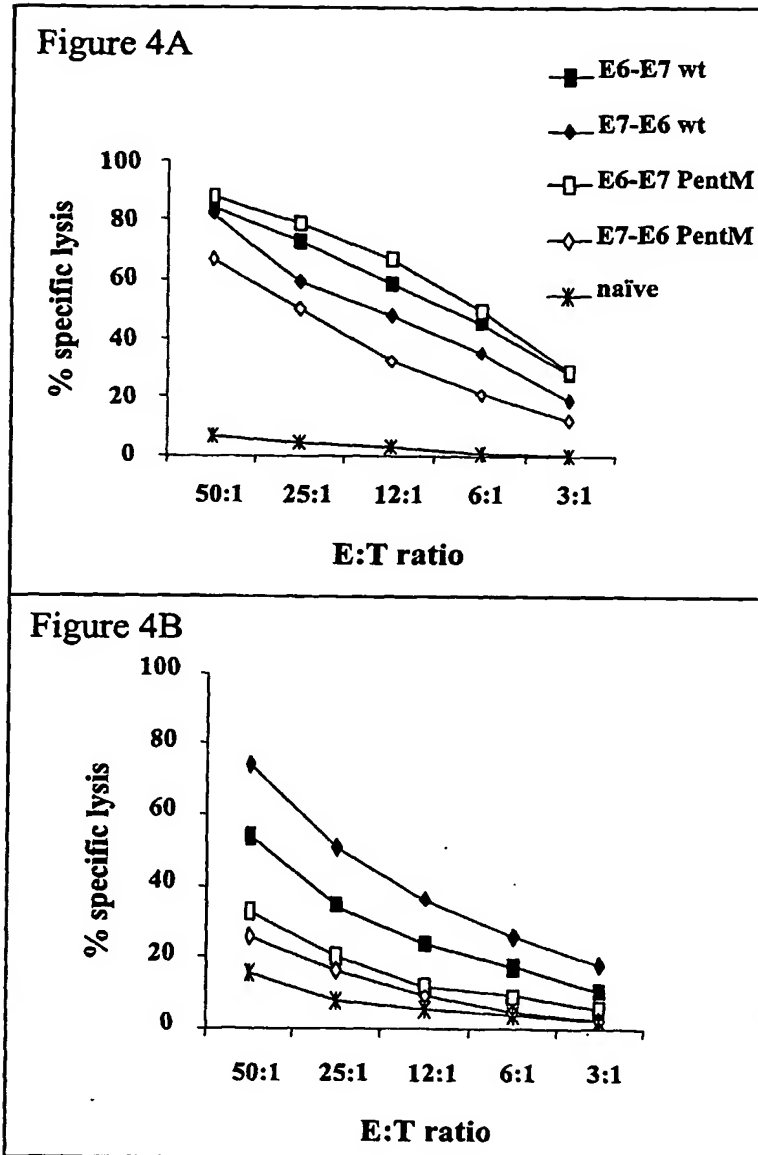


Figure 5

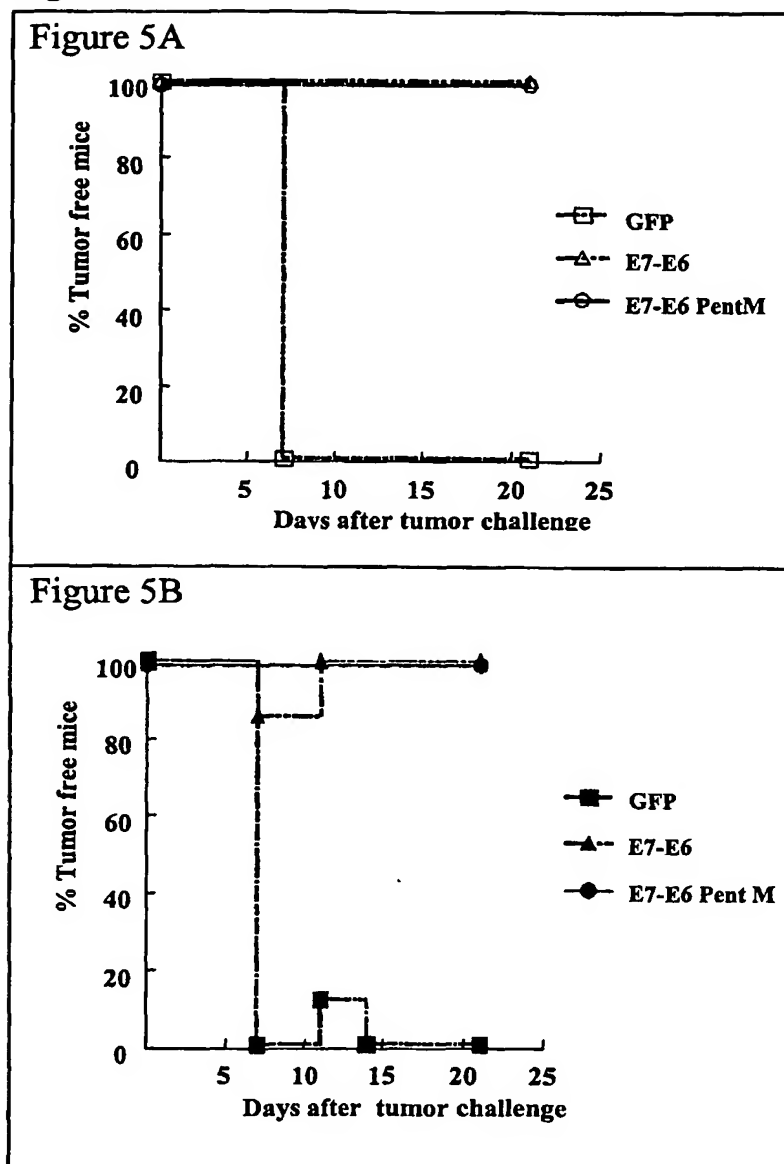


Figure 6

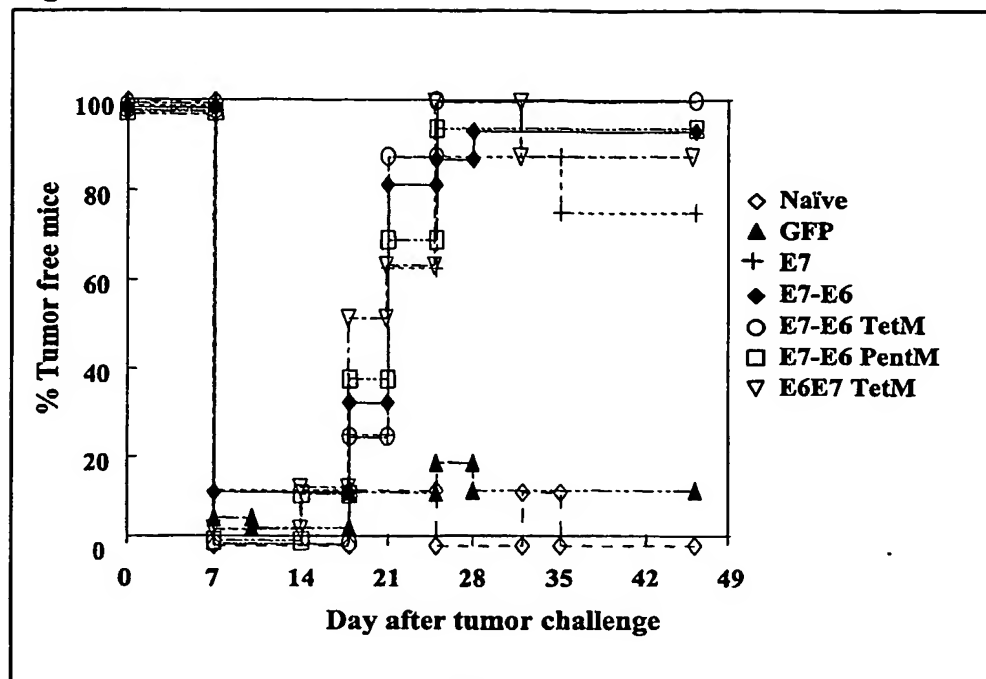


Figure 7

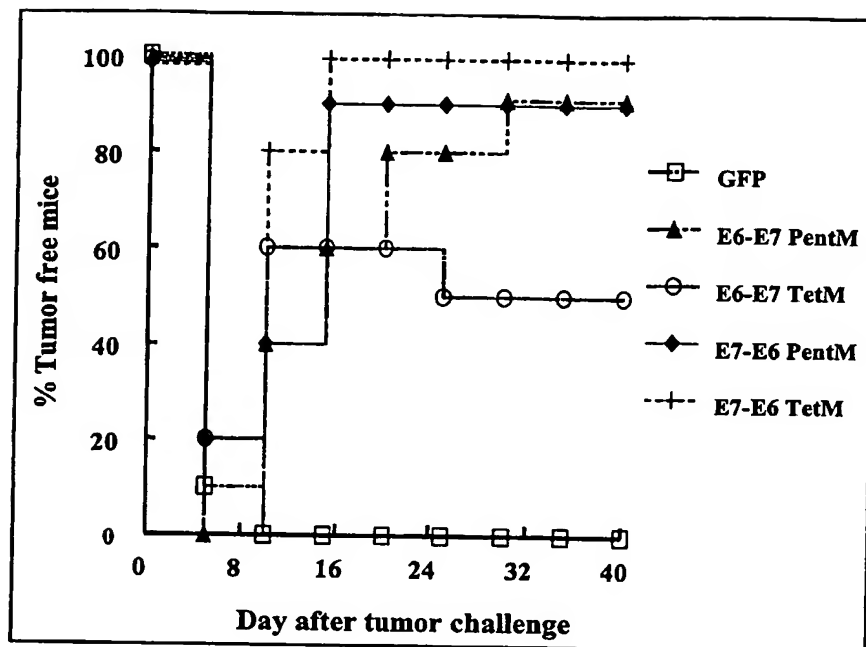
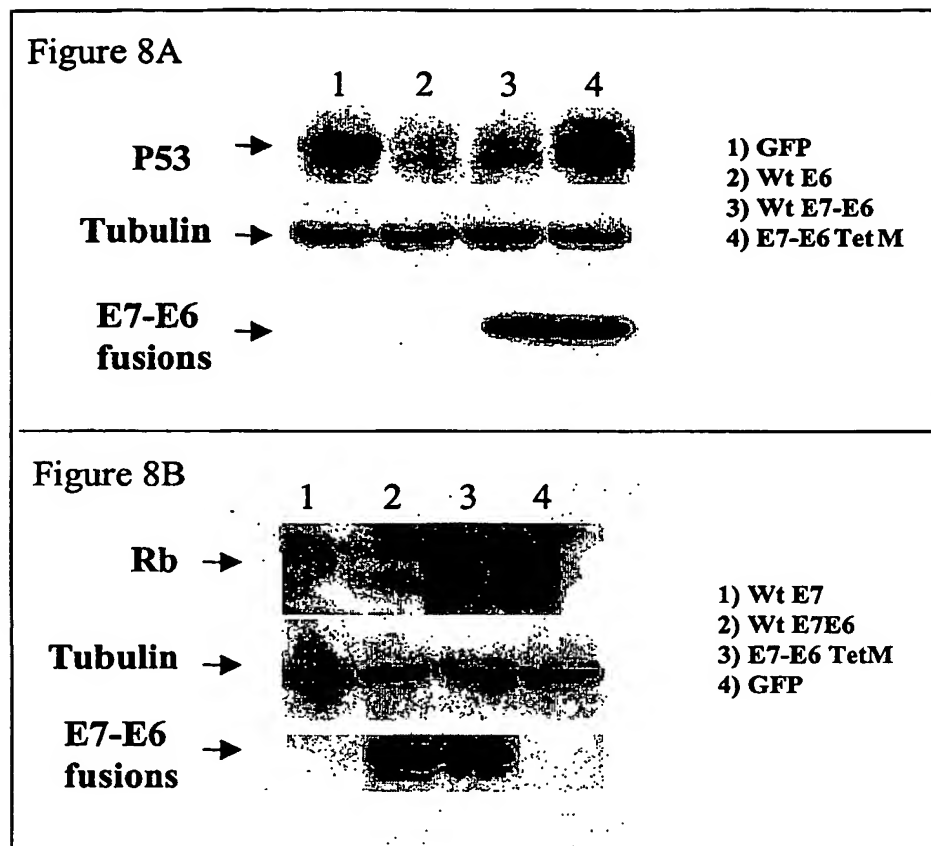


Figure 8



## SEQUENCE LISTING

<110> Wyeth  
Cassetti, Maria C.  
Smith, Larry

<120> HUMAN PAPILLOMAVIRUS POLYPEPTIDES AND IMMUNOGENIC COMPOSITIONS

<130> 00630/200M137-WOO

<150> US 60/415,929

<151> 2002-10-03

<160> 65

<170> PatentIn version 3.1

<210> 1

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Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe Arg  
35 40 45

Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Cys Asp  
50 55 60

Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr Cys  
65 70 75 80

Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro Leu  
85 90 95

Cys Asp Leu Leu Ile Arg Cys Ile Asn Cys Gln Lys Pro Leu Cys Pro  
100 105 110

Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn Ile  
115 120 125

Arg Gly Arg Trp Thr Gly Arg Cys Met Ser Cys Cys Arg Ser Ser Arg  
130 135 140

Thr Arg Arg Glu Thr Gln Leu His Gly Asp Thr Pro Thr Leu His Glu  
145 150 155 160

Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Cys Tyr Glu  
165 170 175

Gln Leu Asn Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala  
 180 185 190

Gly Gln Ala Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys  
 195 200 205

Cys Lys Cys Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val  
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 gaggtatatg actttgcttt tcgggattta tgcatagtat atagagatgg gaatccatat 180  
 gctgtatgtg ataaatgttt aaagttttat tctaaaatta gtgagtatag acattattgt 240  
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 aaaaagcaaa gattccataa tataaggggt cgggtggaccg gtcgatgtat gtcttggtgc 420  
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 cattacaata ttgtaacctt ttgttgcaag tgtgactcta cgcttcggtt gtgcgtacaa 660  
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Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe Arg  
35 40 45

Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Gly Asp  
50 55 60

Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr Cys  
65 70 75 80

Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro Leu  
85 90 95

Cys Asp Leu Leu Ile Arg Cys Ile Asn Gly Gln Lys Pro Leu Cys Pro  
100 105 110

Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn Ile  
115 120 125

Arg Gly Arg Trp Thr Gly Arg Cys Met Ser Cys Cys Arg Ser Ser Arg  
130 135 140

Thr Arg Arg Glu Thr Gln Leu His Gly Asp Thr Pro Thr Leu His Glu  
145 150 155 160

Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly  
165 170 175

Gln Leu Asn Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala  
180 185 190

Gly Gln Ala Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys  
195 200 205

Cys Lys Cys Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val  
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225 230 235 240

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Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe Arg  
 35 40 45

Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Gly Asp  
 50 55 60

Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr Cys  
 65 70 75 80

Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro Leu  
 85 90 95

Cys Asp Leu Leu Ile Arg Cys Ile Asn Gly Gln Lys Pro Leu Cys Pro  
 100 105 110

Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn Ile  
 115 120 125

Arg Gly Arg Trp Thr Gly Arg Cys Met Ser Cys Cys Arg Ser Ser Arg  
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 Thr Arg Arg Glu Thr Gln Leu His Gly Asp Thr Pro Thr Leu His Glu  
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 Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Gly Tyr Gly  
 165 170 175  
 Gln Leu Asn Asp Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala  
 180 185 190  
 Gly Gln Ala Glu Pro Asp Arg Ala His Tyr Asn Ile Val Thr Phe Cys  
 195 200 205  
 Cys Lys Cys Asp Ser Thr Leu Arg Leu Cys Val Gln Ser Thr His Val  
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&lt;212&gt; PRT

&lt;213&gt; Human papillomavirus type 16

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Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr  
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Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu  
65 70 75 80

Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln  
85 90 95

Lys Pro Phe Gln Asp Pro Gln Glu Arg Pro Arg Lys Leu Pro Gln Leu  
100 105 110

Cys Thr Glu Leu Gln Thr Thr Ile His Asp Ile Ile Leu Glu Cys Val  
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Tyr Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe  
130 135 140

Arg Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Cys  
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Asp Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr  
165 170 175

Cys Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro  
180 185 190

Leu Cys Asp Leu Leu Ile Arg Cys Ile Asn Cys Gln Lys Pro Leu Cys  
195 200 205

Pro Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn  
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Arg Thr Arg Arg Glu Thr Gln Leu  
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Glu Glu Glu Asp Glu Ile Asp Gly Pro Ala Gly Gln Ala Glu Pro Asp  
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Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr  
50 55 60

Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu  
65 70 75 80

Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln  
85 90 95

Lys Pro Phe Gln Asp Pro Gln Glu Arg Pro Arg Lys Leu Pro Gln Leu  
 100 105 110

Cys Thr Glu Leu Gln Thr Thr Ile His Asp Ile Ile Leu Glu Cys Val  
 115 120 125

Tyr Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe  
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Arg Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Gly  
 145 150 155 160

Asp Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr  
 165 170 175

Cys Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro  
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Leu Cys Asp Leu Leu Ile Arg Cys Ile Asn Gly Gln Lys Pro Leu Cys  
 195 200 205

Pro Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn  
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Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr  
50 55 60

Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu  
65 70 75 80

Asp Leu Leu Met Gly Thr Leu Gly Ile Val Gly Pro Ile Cys Ser Gln  
85 90 95

Lys Pro Phe Gln Asp Pro Gln Glu Arg Pro Arg Lys Leu Pro Gln Leu  
100 105 110

Cys Thr Glu Leu Gln Thr Thr Ile His Asp Ile Ile Leu Glu Cys Val  
115 120 125

Tyr Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe  
130 135 140

Arg Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Gly  
145 150 155 160

Asp Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr  
165 170 175

Cys Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro  
180 185 190

Leu Cys Asp Leu Leu Ile Arg Cys Ile Asn Gly Gln Lys Pro Leu Cys  
195 200 205

Pro Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn  
 210 215 220

Ile Arg Gly Arg Trp Thr Gly Arg Cys Met Ser Cys Cys Arg Ser Ser  
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Arg Thr Arg Arg Glu Thr Gln Leu  
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Cys Lys Gln Gln Leu Leu Arg Arg Glu Val Tyr Asp Phe Ala Phe Arg  
 35 40 45



Asp Leu Cys Ile Val Tyr Arg Asp Gly Asn Pro Tyr Ala Val Cys Asp  
50 55 60

Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr Cys  
65 70 75 80

Tyr Ser Val Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro Leu  
85 90 95

Cys Asp Leu Leu Ile Arg Cys Ile Asn Cys Gln Lys Pro Leu Cys Pro  
100 105 110

Glu Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn Ile  
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Arg Gly Arg Trp Thr Gly Arg Cys Met Ser Cys Cys Arg Ser Ser Arg  
130 135 140

Thr Arg Arg Glu Thr Gln Leu  
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20 25 30

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35 40 45

Arg Ala His Tyr Asn Ile Val Thr Phe Cys Cys Lys Cys Asp Ser Thr  
50 55 60

Leu Arg Leu Cys Val Gln Ser Thr His Val Asp Ile Arg Thr Leu Glu  
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Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys Pro Ile Cys Ser Gln  
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Lys Pro

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35 40 45

Phe Lys Asp Leu Phe Val Val Tyr Arg Asp Ser Ile Pro His Ala Ala  
50 55 60

Cys His Lys Cys Ile Asp Phe Tyr Ser Arg Ile Arg Glu Leu Arg His  
65 70 75 80

Tyr Ser Asp Ser Val Tyr Gly Asp Thr Leu Glu Lys Leu Thr Asn Thr  
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Gly Leu Tyr Asn Leu Leu Ile Arg Cys Leu Arg Cys Gln Lys Pro Leu  
100 105 110

Asn Pro Ala Glu Lys Leu Arg His Leu Asn Glu Lys Arg Arg Phe His  
115 120 125

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35 40 45

Asp Leu Thr Ile Val Tyr Arg Asp Asp Thr Pro His Gly Val Cys Thr  
50 55 60

Lys Cys Leu Arg Phe Tyr Ser Lys Val Ser Glu Phe Arg Trp Tyr Arg  
65 70 75 80

Tyr Ser Val Tyr Gly Thr Thr Leu Glu Lys Leu Thr Asn Lys Gly Ile  
85 90 95

Cys Asp Leu Leu Ile Arg Cys Ile Thr Cys Gln Arg Pro Leu Cys Pro  
100 105 110

Glu Glu Lys Gln Arg His Leu Asp Lys Lys Lys Arg Phe His Asn Ile  
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Cys Lys Lys Pro Leu Gln Arg Ser Glu Val Tyr Asp Phe Ala Phe Ala  
35 40 45

Asp Leu Thr Val Val Tyr Arg Glu Gly Asn Pro Phe Gly Ile Cys Lys  
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Leu Cys Leu Arg Phe Leu Ser Lys Ile Ser Glu Tyr Arg His Tyr Asn  
65 70 75 80

Tyr Ser Val Tyr Gly Asn Thr Leu Glu Gln Thr Val Lys Lys Pro Leu  
85 90 95

Asn Glu Ile Leu Ile Arg Cys Ile Ile Cys Gln Arg Pro Leu Cys Pro  
100 105 110

Gln Glu Lys Lys Arg His Val Asp Leu Asn Lys Arg Phe His Asn Ile  
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Arg Glu Thr Ala Leu  
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35 40 45

Asp Leu Cys Ile Val Tyr Arg Glu Gly Gln Pro Tyr Gly Val Cys Met  
50 55 60

Lys Cys Leu Lys Phe Tyr Ser Lys Ile Ser Glu Tyr Arg Trp Tyr Arg  
65 70 75 80

Tyr Ser Val Tyr Gly Glu Thr Leu Glu Lys Gln Cys Asn Lys Gln Leu  
85 90 95

Cys His Leu Leu Ile Arg Cys Ile Thr Cys Gln Lys Pro Leu Cys Pro  
100 105 110

Val Glu Lys Gln Arg His Leu Glu Glu Lys Lys Arg Phe His Asn Ile  
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35 40 45

Phe Ser Asp Leu Tyr Val Val Tyr Arg Asp Gly Glu Pro Leu Ala Ala  
50 55 60

Cys Gln Ser Cys Ile Lys Phe Tyr Ala Lys Ile Arg Glu Leu Arg Tyr  
65 70 75 80

Tyr Ser Asp Ser Val Tyr Ala Thr Thr Leu Glu Asn Ile Thr Asn Thr  
85 90 95

Lys Leu Tyr Asn Leu Leu Ile Arg Cys Met Cys Cys Leu Lys Pro Leu  
100 105 110

Cys Pro Ala Glu Lys Leu Arg His Leu Asn Ser Lys Arg Arg Phe His  
115 120 125

Lys Ile Ala Gly Ser Tyr Thr Gly Gln Cys Arg Arg Cys Trp Thr Thr  
130 135 140

Lys Arg Glu Asp Arg Arg Leu Thr Arg Arg Glu Thr Gln Val  
145 150 155

<210> 20  
<211> 158  
<212> PRT  
<213> Human papillomavirus type 45

<400> 20

Met Ala Arg Phe Asp Asp Pro Lys Gln Arg Pro Tyr Lys Leu Pro Asp  
1 5 10 15

Leu Cys Thr Glu Leu Asn Thr Ser Leu Gln Asp Val Ser Ile Ala Cys  
20 25 30

Val Tyr Cys Lys Ala Thr Leu Glu Arg Thr Glu Val Tyr Gln Phe Ala  
35 40 45

Phe Lys Asp Leu Cys Ile Val Tyr Arg Asp Cys Ile Ala Tyr Ala Ala  
50 55 60

Cys His Lys Cys Ile Asp Phe Tyr Ser Arg Ile Arg Glu Leu Arg Tyr  
65 70 75 80

Tyr Ser Asn Ser Val Tyr Gly Glu Thr Leu Glu Lys Ile Thr Asn Thr  
85 90 95

Glu Leu Tyr Asn Leu Leu Ile Arg Cys Leu Arg Cys Gln Lys Pro Leu

100

105

110

Asn Pro Ala Glu Lys Arg Arg His Leu Lys Asp Lys Arg Arg Phe His  
115 120 125

Ser Ile Ala Gly Gln Tyr Arg Gly Gln Cys Asn Thr Cys Cys Asp Gln  
130 135 140

Ala Arg Gln Glu Arg Leu Arg Arg Arg Arg Glu Thr Gln Val  
145 150 155

<210> 21  
<211> 151  
<212> PRT  
<213> Human papillomavirus type 51

<400> 21

Met Phe Glu Asp Lys Arg Glu Arg Pro Arg Thr Leu His Glu Leu Cys  
1 5 10 15

Glu Ala Leu Asn Val Ser Met His Asn Ile Gln Val Val Cys Val Tyr  
20 25 30

Cys Lys Lys Glu Leu Cys Arg Ala Asp Val Tyr Asn Val Ala Phe Thr  
35 40 45

Glu Ile Lys Ile Val Tyr Arg Asp Asn Asn Pro Tyr Ala Val Cys Lys  
50 55 60

Gln Cys Leu Leu Phe Tyr Ser Lys Ile Arg Glu Tyr Arg Arg Tyr Ser  
65 70 75 80

Arg Ser Val Tyr Gly Thr Thr Leu Glu Ala Ile Thr Lys Lys Ser Leu  
85 90 95

Tyr Asp Leu Ser Ile Arg Cys His Arg Cys Gln Arg Pro Leu Gly Pro  
100 105 110

Glu Glu Lys Gln Lys Leu Val Asp Glu Lys Lys Arg Phe His Glu Ile  
115 120 125

Ala Gly Arg Trp Thr Gly Gln Cys Ala Asn Cys Trp Gln Arg Thr Arg  
130 135 140

Gln Arg Asn Glu Thr Gln Val  
145 150

<210> 22  
<211> 148  
<212> PRT  
<213> Human papillomavirus type 52

&lt;400&gt; 22

Met Phe Glu Asp Pro Ala Thr Arg Pro Arg Thr Leu His Glu Leu Cys  
1 5 10 15

Glu Val Leu Glu Glu Ser Val His Glu Ile Arg Leu Gln Cys Val Gln  
20 25 30

Cys Lys Lys Glu Leu Gln Arg Arg Glu Val Tyr Lys Phe Leu Phe Thr  
35 40 45

Asp Leu Arg Ile Val Tyr Arg Asp Asn Asn Pro Tyr Gly Val Cys Ile  
50 55 60

Met Cys Leu Arg Phe Leu Ser Lys Ile Ser Glu Tyr Arg His Tyr Gln  
65 70 75 80

Tyr Ser Leu Tyr Gly Lys Thr Leu Glu Glu Arg Val Lys Lys Pro Leu  
85 90 95

Ser Glu Ile Thr Ile Arg Cys Ile Ile Cys Gln Thr Pro Leu Cys Pro  
100 105 110

Glu Glu Lys Glu Arg His Val Asn Ala Asn Lys Arg Phe His Asn Ile  
115 120 125

Met Gly Arg Trp Thr Gly Arg Cys Ser Glu Cys Trp Arg Pro Arg Pro  
130 135 140

Val Thr Gln Val  
145

&lt;210&gt; 23

&lt;211&gt; 155

&lt;212&gt; PRT

&lt;213&gt; Human papillomavirus type 56

&lt;400&gt; 23

Met Glu Pro Gln Phe Asn Asn Pro Gln Glu Arg Pro Arg Ser Leu His  
1 5 10 15

His Leu Ser Glu Val Leu Glu Ile Pro Leu Ile Asp Leu Arg Leu Ser  
20 25 30

Cys Val Tyr Cys Lys Lys Glu Leu Thr Arg Ala Glu Val Tyr Asn Phe  
35 40 45

Ala Cys Thr Glu Leu Lys Leu Val Tyr Arg Asp Asp Phe Pro Tyr Ala  
50 55 60

Val Cys Arg Val Cys Leu Leu Phe Tyr Ser Lys Val Arg Lys Tyr Arg  
65 70 75 80

Tyr Tyr Asp Tyr Ser Val Tyr Gly Ala Thr Leu Glu Ser Ile Thr Lys  
85 90 95

Lys Gln Leu Cys Asp Leu Leu Ile Arg Cys Tyr Arg Cys Gln Ser Pro  
100 105 110

Leu Thr Pro Glu Glu Lys Gln Leu His Cys Asp Arg Lys Arg Arg Phe  
115 120 125

His Leu Ile Ala His Gly Trp Thr Gly Ser Cys Leu Gly Cys Trp Arg  
130 135 140

Gln Thr Ser Arg Glu Pro Arg Glu Ser Thr Val  
145 150 155

<210> 24  
<211> 149  
<212> PRT  
<213> Human papillomavirus type 58

<400> 24

Met Phe Gln Asp Ala Glu Glu Lys Pro Arg Thr Leu His Asp Leu Cys  
1 5 10 15

Gln Ala Leu Glu Thr Ser Val His Glu Ile Glu Leu Lys Cys Val Glu  
20 25 30

Cys Lys Lys Thr Leu Gln Arg Ser Glu Val Tyr Asp Phe Val Phe Ala  
35 40 45

Asp Leu Arg Ile Val Tyr Arg Asp Gly Asn Pro Phe Ala Val Cys Lys  
50 55 60

Val Cys Leu Arg Leu Leu Ser Lys Ile Ser Glu Tyr Arg His Tyr Asn  
65 70 75 80

Tyr Ser Leu Tyr Gly Asp Thr Leu Glu Gln Thr Leu Lys Lys Cys Leu  
85 90 95

Asn Glu Ile Leu Ile Arg Cys Ile Ile Cys Gln Arg Pro Leu Cys Pro  
100 105 110

Gln Glu Lys Lys Arg His Val Asp Leu Asn Lys Arg Phe His Asn Ile  
115 120 125

Ser Gly Arg Trp Thr Gly Arg Cys Ala Val Cys Trp Arg Pro Arg Arg  
130 135 140



Arg Gln Thr Gln Val  
145

<210> 25  
<211> 160  
<212> PRT  
<213> Human papillomavirus type 59

<400> 25

Met Ala Arg Phe Glu Asp Pro Thr Gln Arg Pro Tyr Lys Leu Pro Asp  
1 5 10 15

Leu Ser Thr Thr Leu Asn Ile Pro Leu His Asp Ile Arg Ile Asn Cys  
20 25 30

Val Phe Cys Lys Gly Glu Leu Gln Glu Arg Glu Val Phe Glu Phe Ala  
35 40 45

Phe Asn Asp Leu Phe Ile Val Tyr Arg Asp Cys Thr Pro Tyr Ala Ala  
50 55 60

Cys Leu Lys Cys Ile Ser Phe Tyr Ala Arg Val Arg Glu Leu Arg Tyr  
65 70 75 80

Tyr Arg Asp Ser Val Tyr Gly Glu Thr Leu Glu Ala Glu Thr Lys Thr  
85 90 95

Pro Leu His Glu Leu Leu Ile Arg Cys Tyr Arg Cys Leu Lys Pro Leu  
100 105 110

Cys Pro Thr Asp Lys Leu Lys His Ile Thr Glu Lys Arg Arg Phe His  
115 120 125

Asn Ile Ala Gly Ile Tyr Thr Gly Gln Cys Arg Gly Cys Arg Thr Arg  
130 135 140

Ala Arg His Leu Arg Gln Gln Arg Gln Ala Arg Ser Glu Thr Leu Val  
145 150 155 160

<210> 26  
<211> 158  
<212> PRT  
<213> Human papillomavirus type 68

<400> 26

Met Ala Leu Phe His Asn Pro Glu Glu Arg Pro Tyr Lys Leu Pro Asp  
1 5 10 15

Leu Cys Arg Thr Leu Asp Thr Thr Leu His Asp Val Thr Ile Asp Cys  
20 25 30

Val Tyr Cys Arg Arg Gln Leu Gln Arg Thr Glu Val Tyr Glu Phe Ala  
35 40 45

Phe Ser Asp Leu Cys Val Val Tyr Arg Asp Gly Val Pro Phe Ala Ala  
50 55 60

Cys Gln Ser Cys Ile Lys Phe Tyr Ala Lys Ile Arg Glu Leu Arg Tyr  
65 70 75 80

Tyr Ser Glu Ser Val Tyr Ala Thr Thr Leu Glu Thr Ile Thr Asn Thr  
85 90 95

Lys Leu Tyr Asn Leu Leu Ile Arg Cys Met Ser Cys Leu Lys Pro Leu  
100 105 110

Cys Pro Ala Glu Lys Leu Arg His Leu Thr Thr Lys Arg Arg Leu His  
115 120 125

Lys Ile Ala Gly Asn Phe Thr Gly Gln Cys Arg His Cys Trp Thr Ser  
130 135 140

Lys Arg Glu Asp Arg Arg Arg Ile Arg Gln Glu Thr Gln Val  
145 150 155

<210> 27

<211> 105

<212> PRT

<213> Human papillomavirus type 18

<400> 27

Met His Gly Pro Lys Ala Thr Leu Gln Asp Ile Val Leu His Leu Glu  
1 5 10 15

Pro Gln Asn Glu Ile Pro Val Asp Leu Leu Cys His Glu Gln Leu Ser  
20 25 30

Asp Ser Glu Glu Glu Asn Asp Glu Ile Asp Gly Val Asn His Gln His  
35 40 45

Leu Pro Ala Arg Arg Ala Glu Pro Gln Arg His Thr Met Leu Cys Met  
50 55 60

Cys Cys Lys Cys Glu Ala Arg Ile Lys Leu Val Val Glu Ser Ser Ala  
65 70 75 80

Asp Asp Leu Arg Ala Phe Gln Gln Leu Phe Leu Asn Thr Leu Ser Phe  
85 90 95

Val Cys Pro Trp Cys Ala Ser Gln Gln  
100 105

<210> 28  
<211> 98  
<212> PRT  
<213> Human papillomavirus type 31

<400> 28

Met Arg Gly Glu Thr Pro Thr Leu Gln Asp Tyr Val Leu Asp Leu Gln  
1 5 10 15

Pro Glu Ala Thr Asp Leu His Cys Tyr Glu Gln Leu Pro Asp Ser Ser  
20 25 30

Asp Glu Glu Asp Val Ile Asp Ser Pro Ala Gly Gln Ala Glu Pro Asp  
35 40 45

Thr Ser Asn Tyr Asn Ile Val Thr Phe Cys Cys Gln Cys Lys Ser Thr  
50 55 60

Leu Arg Leu Cys Val Gln Ser Thr Gln Val Asp Ile Arg Ile Leu Gln  
65 70 75 80

Glu Leu Leu Met Gly Ser Phe Gly Ile Val Cys Pro Asn Cys Ser Thr  
85 90 95

Arg Leu

<210> 29  
<211> 97  
<212> PRT  
<213> Human papillomavirus type 33

<400> 29

Met Arg Gly His Lys Pro Thr Leu Lys Glu Tyr Val Leu Asp Leu Tyr  
1 5 10 15

Pro Glu Pro Thr Asp Leu Tyr Cys Tyr Glu Gln Leu Ser Asp Ser Ser  
20 25 30

Asp Glu Asp Glu Gly Leu Asp Arg Pro Asp Gly Gln Ala Gln Pro Ala  
35 40 45

Thr Ala Asp Tyr Tyr Ile Val Thr Cys Cys His Thr Cys Asn Thr Thr  
50 55 60

Val Arg Leu Cys Val Asn Ser Thr Ala Ser Asp Leu Arg Thr Ile Gln  
65 70 75 80

Gln Leu Leu Met Gly Thr Val Asn Ile Val Cys Pro Thr Cys Ala Gln  
85 90 95

Gln

<210> 30  
<211> 99  
<212> PRT  
<213> Human papillomavirus type 35

&lt;400&gt; 30

Met His Gly Glu Ile Thr Thr Leu Gln Asp Tyr Val Leu Asp Leu Glu  
1 5 10 15

Pro Glu Ala Thr Asp Leu Tyr Cys Tyr Glu Gln Leu Cys Asp Ser Ser  
20 25 30

Glu Glu Glu Glu Asp Thr Ile Asp Gly Pro Ala Gly Gln Ala Lys Pro  
35 40 45

Asp Thr Ser Asn Tyr Asn Ile Val Thr Ser Cys Cys Lys Cys Glu Ala  
50 55 60

Thr Leu Arg Leu Cys Val Gln Ser Thr His Ile Asp Ile Arg Lys Leu  
65 70 75 80

Glu Asp Leu Leu Met Gly Thr Phe Gly Ile Val Cys Pro Gly Cys Ser  
85 90 95

Gln Arg Ala

<210> 31  
<211> 109  
<212> PRT  
<213> Human papillomavirus type 39

&lt;400&gt; 31

Met Arg Gly Pro Lys Pro Thr Leu Gln Glu Ile Val Leu Asp Leu Cys  
1 5 10 15

Pro Tyr Asn Glu Ile Gln Pro Val Asp Leu Val Cys His Glu Gln Leu  
20 25 30

Gly Glu Ser Glu Asp Glu Ile Asp Glu Pro Asp His Ala Val Asn His  
35 40 45

Gln His Gln Leu Leu Ala Arg Arg Asp Glu Pro Gln Arg His Thr Ile  
50 55 60

Gln Cys Ser Cys Cys Lys Cys Asn Asn Thr Leu Gln Leu Val Val Glu  
65 70 75 80

Ala Ser Arg Asp Thr Leu Arg Gln Leu Gln Gln Leu Phe Met Asp Ser  
85 90 95

Leu Gly Phe Val Cys Pro Trp Cys Ala Thr Ala Asn Gln  
100 105

<210> 32  
<211> 106  
<212> PRT  
<213> Human papillomavirus type 45  
<400> 32

Met His Gly Pro Arg Glu Thr Leu Gln Glu Ile Val Leu His Leu Glu  
1 5 10 15

Pro Gln Asn Glu Leu Asp Pro Val Asp Leu Leu Cys Tyr Glu Gln Leu  
20 25 30

Ser Glu Ser Glu Glu Glu Asn Asp Glu Ala Asp Gly Val Ser His Ala  
35 40 45

Gln Leu Pro Ala Arg Arg Ala Glu Pro Gln Arg His Lys Ile Leu Cys  
50 55 60

Val Cys Cys Lys Cys Asp Gly Arg Ile Glu Leu Thr Val Glu Ser Ser  
65 70 75 80

Ala Glu Asp Leu Arg Thr Leu Gln Gln Leu Phe Leu Ser Thr Leu Ser  
85 90 95

Phe Val Cys Pro Trp Cys Ala Thr Asn Gln  
100 105

<210> 33  
<211> 101  
<212> PRT  
<213> Human papillomavirus type 51  
<400> 33

Met Arg Gly Asn Val Pro Gln Leu Lys Asp Val Val Leu His Leu Thr  
1 5 10 15

Pro Gln Thr Glu Ile Asp Leu Gln Cys Tyr Glu Gln Phe Asp Ser Ser  
20 25 30

Glu Glu Glu Asp Glu Val Asp Asn Met Arg Asp Gln Leu Pro Glu Arg  
35 40 45

Arg Ala Gly Gln Ala Thr Cys Tyr Arg Ile Glu Ala Pro Cys Cys Arg  
50 55 60

Cys Ser Ser Val Val Gln Leu Ala Val Glu Ser Ser Gly Asp Thr Leu  
65 70 75 80

Arg Val Val Gln Gln Met Leu Met Gly Glu Leu Ser Leu Val Cys Pro  
85 90 95

Cys Cys Ala Asn Asn  
100

<210> 34  
<211> 99  
<212> PRT  
<213> Human papillomavirus type 52

<400> 34

Met Arg Gly Asp Lys Ala Thr Ile Lys Asp Tyr Ile Leu Asp Leu Gln  
1 5 10 15

Pro Glu Thr Thr Asp Leu His Cys Tyr Glu Gln Leu Gly Asp Ser Ser  
20 25 30

Asp Glu Glu Asp Thr Asp Gly Val Asp Arg Pro Asp Gly Gln Ala Glu  
35 40 45

Gln Ala Thr Ser Asn Tyr Tyr Ile Val Thr Tyr Cys His Ser Cys Asp  
50 55 60

Ser Thr Leu Arg Leu Cys Ile His Ser Thr Ala Thr Asp Leu Arg Thr  
65 70 75 80

Leu Gln Gln Met Leu Leu Gly Thr Leu Gln Val Val Cys Pro Gly Cys  
85 90 95

Ala Arg Leu

<210> 35  
<211> 105  
<212> PRT  
<213> Human papillomavirus type 56

<400> 35

Met His Gly Lys Val Pro Thr Leu Gln Asp Val Val Leu Glu Leu Thr  
1 5 10 15

Pro Gln Thr Glu Ile Asp Leu Gln Cys Asn Glu Gln Leu Asp Ser Ser  
20 25 30

Glu Asp Glu Asp Glu Asp Glu Val Asp His Leu Gln Glu Arg Pro Gln  
35 40 45

Gln Ala Arg Gln Ala Lys Gln His Thr Cys Tyr Leu Ile His Val Pro  
50 55 60

Cys Cys Glu Cys Lys Phe Val Val Gln Leu Asp Ile Gln Ser Thr Lys  
65 70 75 80

Glu Asp Leu Arg Val Val Gln Gln Leu Leu Met Gly Ala Leu Thr Val  
85 90 95

Thr Cys Pro Leu Cys Ala Ser Ser Asn  
100 105

<210> 36  
<211> 98  
<212> PRT  
<213> Human papillomavirus type 58

<400> 36

Met Arg Gly Asn Asn Pro Thr Leu Arg Glu Tyr Ile Leu Asp Leu His  
1 5 10 15

Pro Glu Pro Thr Asp Leu Phe Cys Tyr Glu Gln Leu Cys Asp Ser Ser  
20 25 30

Asp Glu Asp Glu Ile Gly Leu Asp Gly Pro Asp Gly Gln Ala Gln Pro  
35 40 45

Ala Thr Ala Asn Tyr Tyr Ile Val Thr Cys Cys Tyr Thr Cys Gly Thr  
50 55 60

Thr Val Arg Leu Cys Ile Asn Ser Thr Thr Thr Asp Val Arg Thr Leu  
65 70 75 80

Gln Gln Leu Leu Met Gly Thr Cys Thr Ile Val Cys Pro Ser Cys Ala  
85 90 95

Gln Gln

<210> 37  
<211> 107  
<212> PRT  
<213> Human papillomavirus type 59

<400> 37

Met His Gly Pro Lys Ala Thr Leu Cys Asp Ile Val Leu Asp Leu Glu  
1 5 10 15

Pro Gln Asn Tyr Glu Glu Val Asp Leu Val Cys Tyr Glu Gln Leu Pro  
20 25 30

Asp Ser Asp Ser Glu Asn Glu Lys Asp Glu Pro Asp Gly Val Asn His  
35 40 45

Pro Leu Leu Leu Ala Arg Arg Ala Glu Pro Gln Arg His Asn Ile Val  
50 55 60

Cys Val Cys Cys Lys Cys Asn Asn Gln Leu Gln Leu Val Val Glu Thr  
65 70 75 80

Ser Gln Asp Gly Leu Arg Ala Leu Gln Gln Leu Phe Met Asp Thr Leu  
85 90 95

Ser Phe Val Cys Pro Leu Cys Ala Ala Asn Gln  
100 105

<210> 38  
<211> 110  
<212> PRT  
<213> Human papillomavirus type 68

<400> 38

Met His Gly Pro Lys Pro Thr Val Gln Glu Ile Val Leu Glu Leu Cys  
1 5 10 15

Pro Tyr Asn Glu Ile Gln Pro Val Asp Leu Val Cys His Glu Gln Leu  
20 25 30

Gly Asp Ser Asp Asp Glu Ile Asp Glu Pro Asp His Ala Val Asn His  
35 40 45

His Gln His Leu Leu Leu Ala Arg Arg Asp Glu Gln Gln Arg His Arg  
50 55 60

Ile Gln Cys Leu Cys Cys Lys Cys Asn Lys Ala Leu Gln Leu Val Val  
65 70 75 80

Glu Ala Ser Arg Asp Asn Leu Arg Thr Leu Gln Gln Leu Phe Met Asp  
85 90 95

Ser Leu Asn Phe Val Cys Pro Trp Cys Ala Thr Glu Thr Gln  
100 105 110

<210> 39  
<211> 152  
<212> PRT  
<213> Human papillomavirus

<220>  
<221> MISC\_FEATURE  
<222> (1)..(152)  
<223> where Xaa is any amino acid



&lt;400&gt; 39

Xaa Xaa Xaa Phe Glx Asp Pro Xaa Glu Arg Pro Xaa Lys Leu Xaa Asp  
1 5 10 15

Leu Cys Xaa Xaa Leu Xaa Xaa Xaa Xaa His Asx Ile Xaa Xaa Xaa Cys  
20 25 30

Val Tyr Cys Lys Xaa Glx Leu Glx Arg Xaa Glu Val Tyr Xaa Phe Ala  
35 40 45

Phe Xaa Asp Leu Xaa Ile Val Tyr Arg Asp Xaa Xaa Pro Xaa Ala Xaa  
50 55 60

Cys Xaa Xaa Cys Leu Xaa Phe Tyr Ser Lys Ile Xaa Glu Xaa Arg Xaa  
65 70 75 80

Tyr Xaa Xaa Ser Val Tyr Gly Xaa Thr Leu Glu Xaa Xaa Thr Xaa Lys  
85 90 95

Xaa Leu Xaa Asx Leu Leu Ile Arg Cys Xaa Xaa Cys Gln Xaa Pro Leu  
100 105 110

Cys Pro Xaa Glu Lys Xaa Arg His Xaa Asx Xaa Lys Xaa Arg Phe His  
115 120 125

Asn Ile Xaa Gly Xaa Trp Thr Gly Xaa Cys Xaa Xaa Cys Trp Xaa Xaa  
130 135 140

Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa  
145 150

&lt;210&gt; 40

&lt;211&gt; 104

&lt;212&gt; PRT

&lt;213&gt; Human papillomavirus

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (1)..(104)

&lt;223&gt; where Xaa is any amino acid

&lt;400&gt; 40

Met Xaa Gly Xaa Xaa Pro Thr Leu Xaa Xaa Xaa Val Leu Asp Leu Xaa  
1 5 10 15

Pro Glx Xaa Xaa Xaa Asp Leu Xaa Cys Tyr Glu Gln Leu Xaa Asp Ser  
20 25 30

Xaa Xaa Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

35

40

45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Xaa Ile Xaa Xaa Xaa Cys  
50 55 60

Cys Xaa Cys Xaa Xaa Xaa Xaa Xaa Leu Xaa Val Glx Ser Xaa Xaa Xaa  
65 70 75 80

Asp Leu Arg Xaa Leu Gln Gln Leu Leu Met Gly Thr Leu Xaa Xaa Val  
85 90 95

Cys Pro Xaa Cys Ala Xaa Xaa Xaa  
100

<210> 41  
<211> 9  
<212> PRT  
<213> Human papillomavirus type 16

<400> 41

Arg Ala His Tyr Asn Ile Val Thr Phe  
1 5

<210> 42  
<211> 477  
<212> DNA  
<213> Human papillomavirus type 18

<400> 42  
atggcgcgct ttgaggatcc aacacggcga ccctacaagc tacctgatct gtgcacggaa 60  
ctgaacactt cactgcaaga catagaaata acctgtgtat attgcaagac agtattggaa 120  
cttacagagg tatTTgaatt tgcatttaaa gatttatttg tgggtgtatag agacagtata 180  
cccatgctg catgccataa atgtatagat ttttattcta gaattagaga attaagacat 240  
tattcagact ctgtgtatgg agacacattg gaaaaactaa ctaacactgg gttatacaat 300  
ttattaataa ggtgcctgcg gtgccagaaa ccgttgaatc cagcagaaaa acttagacac 360  
cttaatgaaa aacgacgatt tcacaacata gctgggcact atagaggcca gtgccattcg 420  
tgctgcaacc gagcacgaca ggaacgactc caacgacgca gagaaacaca agtataa 477

<210> 43  
<211> 474  
<212> DNA  
<213> Human papillomavirus type 31

<400> 43  
atgttcaaaa atcctgcaga aagacctcgg aaattgcatg aactaagctc ggcattggaa 60  
ataccctacg atgaactaag attgaattgt gtctactgca aaggtcagtt aacagaaaca 120  
gaggtattag attttgcatt tacagattta acaatagtat atagggacga cacaccacac 180  
ggagtgtgta caaaatgttt aagattttat tcaaaagtaa gtgaatttag atggtataga 240

tatagtgtgt atggaacaac attagaaaaa ttgacaaaca aaggtatatg tgatttggtta 300  
attaggtgta taacgtgtca aagaccgttg tgtccagaag aaaaacaaag acatttggat 360  
aaaaagaaac gattccacaa cataggagga aggtggacag gacgttgcac agcatgttgg 420  
agaagacctc gtactgaaac ccaagtgtaa acatgcgtgg agaaacacct acgt 474

<210> 44  
<211> 450  
<212> DNA  
<213> Human papillomavirus type 33

<400> 44  
atgtttcaag acactgagga aaaaccacga acattgcatg atttgtgcca agcattggag 60  
acaactatac acaacattga actacagtgc gtggaatgca aaaaaccttt gcaacgatct 120  
gaggtatatg attttgcatt tgcagattta acagttgtat atagagaggg aaatccattt 180  
ggaatatgta aactgtgttt gcggttctta tctaaaatta gtgaatatag acattataat 240  
tattctgtat atggaaatac attagaacaa acagttaaaa aacctttaaa tgaaatatta 300  
attaggtgta ttatatgtca aagacctttg tgtcctcaag aaaaaaacg acatgtggat 360  
ttaaacaaac gatttcataa tatttcgggt cgttgggcag ggcgctgtgc ggcgtgttgg 420  
aggtcccgac gtagagaaac tgcactgtga 450

<210> 45  
<211> 450  
<212> DNA  
<213> Human papillomavirus type 35

<400> 45  
atgtttcagg acccagctga acgaccttac aaactgcatg atttgtgcaa cgaggtagaa 60  
gaaagcatcc atgaaatttg tttgaattgt gtatactgca aacaagaatt acagcggagt 120  
gaggtatatg actttgcatg ctatgatttg tgtatagtat atagagaagg ccagccatat 180  
ggagtatgca tgaaatgttt aaaattttat tcaaaaataa gtgaatatag atggatataga 240  
tatagtgtgt atggagaaac gttagaaaaa caatgcaaca aacagttatg tcatttatta 300  
attaggtgta ttacatgtca aaaaccgctg tgtccagttg aaaagcaaag acatttagaa 360  
gaaaaaaaaac gattccataa catcgggtgga cgggtggacag gtcggtgtat gtcctgttgg 420  
aaaccaacac gtagagaaac cgaggtgtaa 450

<210> 46  
<211> 477  
<212> DNA  
<213> Human papillomavirus type 39

<400> 46  
atggcgcgat ttcacaatcc tgcagaacgg ccatacaaat tgccagacct gtgcacaacg 60  
ctggacacca ccttgcagga cattacaata gcctgtgtct attgcagacg accactacag 120

caaaccgagg tatatgaatt tgcatttagt gattttatatg tagtatatag ggacggggaa 180  
ccactagctg catgccaatc atgtataaaa ttttatgcta aaatacggga gctacgatat 240  
tactcggact cgggtgatgc aactacatta gaaaatataa ctaatacaaa gttatataat 300  
ttattaataa ggtgcatgtg ttgtctgaaa ccgctgtgtc cagcagaaaa attaagacac 360  
ctaaatagca aacgaagatt tcataaaata gcaggaagct atacaggaca gtgtcgacgg 420  
tgctggacca caaaacggga ggaccgcaga ctaacacgaa gagaaaccca agtataa 477

<210> 47  
<211> 477  
<212> DNA  
<213> Human papillomavirus type 45

<400> 47  
atggcgcgct ttgacgatcc aaagcaacga ccctacaagc taccagattt gtgcacagaa 60  
ttgaatacat cactacaaga cgtatctatt gcctgtgtat attgcaaagc aacattggaa 120  
cgcacagagg tatatcaatt tgctttttaa gattttatgta tagtgtatag agactgtata 180  
gcatatgctg catgccataa atgtatagac ttttattcca gaattagaga attaagatat 240  
tattcaaact ctgtatatgg agagacactg gaaaaataa ctaatacaga gttgtataat 300  
ttgttaataa ggtgcctgcg gtgccagaaa ccattgaacc cagcagaaaa acgtagacac 360  
cttaaggaca aacgaagatt tcacagcata gctggacagt accgagggca gtgtaataca 420  
tgttgtgacc aggcacggca agaaagactt cgcagacgta gggaaacaca agtatag 477

<210> 48  
<211> 456  
<212> DNA  
<213> Human papillomavirus type 51

<400> 48  
atgttcgaag acaagaggga aagaccacga acgctgcatg aattatgtga agctttgaac 60  
gtttctatgc acaatataca ggtagtgtgt gtgtattgta aaaaggaatt atgtagagca 120  
gatgtatata atgtagcatt tactgaaatt aagattgtat atagggataa taatccatat 180  
gcagtatgca aacaatgttt actgttttat tcaaaaatta gagagtatag acgttatagc 240  
aggtctgtgt atggtactac attagaggca attactaaaa aaagcttata tgatttatcg 300  
ataaggtgtc atagatgtca aagaccactt gggcctgaag aaaagcaaaa attggtggac 360  
gaaaaaaaaa ggttccatga aatagcggga cgttggacgg ggcaatgcgc taattgctgg 420  
caacgtacac gacaacgtaa cgaaacccaa gtgtaa 456

<210> 49  
<211> 447  
<212> DNA  
<213> Human papillomavirus type 52

<400> 49  
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gaatcgggtgc atgaaataag gctgcagtgt gtgcagtgc aaaaagagct acaacgaaga 120  
gaggatataca agttttctatt tacagattta cgaatagtat atagagacaa taatccatat 180  
ggcgtgtgta ttatgtgcct acgcttttta tctaagataa gtgaatatag gcattatcaa 240  
tattcactgt atgggaaaac attagaagag agggtaaaaa aaccattaag tgaaataact 300  
attagatgta taatttgtca aacgccatta tgtcctgaag aaaaagaaag acatgttaat 360  
gcaaacaagc gatttcataa tattatgggt cgttggacag ggcgctgttc agagtgttgg 420  
agaccccgac ctgtgaccca agtgttaa 447

<210> 50  
<211> 465  
<212> DNA  
<213> Human papillomavirus type 56

<400> 50  
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gtattagaaa tacctttaat tgatcttaga ttatcatgtg tatattgcaa aaaagaacta 120  
acacgtgctg aggtatataa ttttgcattg actgaattaa aattagtgtg tagggatgat 180  
tttcttatg cagtgtgcag agtatgttta ttgttttata gttaaagttag aaaatatagg 240  
tattatgact attcagtgtg tggagctaca ctagaaagta taactaaaaa acagttatgt 300  
gatttattaa taagggtgcta cagatgtcaa agtccgttaa ctccggagga aaagcaattg 360  
cattgtgaca gaaaaagacg atttcatcta atagcacatg gttggaccgg gtcattgtttg 420  
gggtgctgga gacaaacatc tagagaacct agagaatcta cagta 465

<210> 51  
<211> 450  
<212> DNA  
<213> Human papillomavirus type 58

<400> 51  
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acatctgtgc atgaaatcga attgaaatgc gttgaatgca aaaagacttt gcagcgatct 120  
gaggatatatg actttgtatt tgcagattta agaatagtgt atagagatgg aaatccattt 180  
gcagtatgta aagtgtgctt acgattgcta tctaaaataa gtgagtatag acattataat 240  
tattcgctat atggagacac attagaacaa aactaaaaa agtgttttaa tgaaatatta 300  
attagatgta ttatttgtca aagaccattg tgtccacaag aaaaaaaaag gcatgtggat 360  
ttaaacaaaa ggtttcataa tatttcgggt cgttggacag ggcgctgtgc agtgtgttgg 420  
agaccccgac gtagacaaac acaagtgtaa 450

<210> 52  
<211> 483  
<212> DNA  
<213> Human papillomavirus type 59

<400> 52  
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ttgaatatct ctctgcatga tattcgcatc aattgtgtgt tttgcaaagg ggaactgcaa 120  
gaaagagagg tatttgaatt tgcttttaat gacttattta tagtgtatag agactgtaca 180  
ccgtatgcag cgtgtctgaa atgcatttca ttttatgcaa gagtaagaga attaagatat 240  
tatagagatt ccgtgtatgg agaaacatta gaggctgaaa ccaagacacc gttacatgag 300  
ctgctgatac gctgttatag atgcctaaaa cctctatgtc caacagataa attaaagcat 360  
ataactgaaa aaagaagatt ccataatata gctggaatat atacaggaca gtgtcgtggg 420  
tgtcggaccc gagcaagaca cctaagacag caacgacaag cgcgtagtga aacactggtg 480  
taa 483

<210> 53  
<211> 477  
<212> DNA  
<213> Human papillomavirus type 68

<400> 53  
atggcgctat ttcacaaccc tgaggaacgg ccatacaaat tgccagacct gtgcaggaca 60  
ttggacacta cattgcatga cgttacaata gactgtgtct attgcagaag gcaactacaa 120  
cggacagagg tatatgaatt tgcccttagt gacctatgtg tagtgtatag agacggggta 180  
ccatttgctg catgccaatc atgtattaaa ttttatgcta aaatacggga actacgatat 240  
tactcggaat cgggtgtatgc aactacatta gaaaccataa ctaatacaaa gttatataat 300  
ttattgataa ggtgcatgag ttgcctgaaa ccatttgtgtc cagcagaaaa actaaggcac 360  
ctaacaacaa aacgaagatt acataaaata gcaggaaact ttacaggaca gtgtcggcac 420  
tgctggacca gtaagcgaga ggaccgcaga cgcatacgtc aagaaacaca agtttaa 477

<210> 54  
<211> 318  
<212> DNA  
<213> Human papillomavirus type 18

<400> 54  
atgcatggac ctaaggcaac attgcaagac attgtattgc atttagagcc ccaaaatgaa 60  
attccggttg accttctatg tcacgagcaa ttaagcgact cagaggaaga aaacgatgaa 120  
atagatggag ttaatcatca acattttacca gcccgcagag ccgaaccaca acgtcacaca 180  
atgttgtgta tgtgttgtaa gtgtgaagcc agaattaagc tagtagtaga aagctcagca 240  
gacgaccttc gagcattcca gcagctgttt ctgaacaccc tgtcctttgt gtgtccgtgg 300  
tgtgcatccc agcagtaa 318

<210> 55  
<211> 297  
<212> DNA

<213> Human papillomavirus type 31

<400> 55  
atgCGTggag aaacacctac gttgcaagac tatgtgtag atttgcaacc tgaggcaact 60  
gacctccact gttatgagca attacccgac agctcagatg aggaggatgt catagacagt 120  
ccagctggac aagcagaacc ggacacatcc aattacaata tcgttacctt ttgttgtcag 180  
tgtaagtcta cacttcgttt gtgtgtacag agcacacaag tagatattcg catattgcaa 240  
gagctgttaa tggggtcatt tggaatcgtg tgccccaact gttctactag actgtaa 297

<210> 56

<211> 294

<212> DNA

<213> Human papillomavirus type 33

<400> 56  
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gacctatact gctatgagca attaagtac agctcagatg aggatgaagg cttggaccgg 120  
ccagatggac aagcacaacc agccacagct gattactaca ttgtaacctg ttgtcacact 180  
tgtaacacca cagttcgttt atgtgtcaac agtacagcaa gtgacctacg aaccatacag 240  
caactactta tgggcacagt gaatattgtg tgccctacct gtgcacaaca ataa 294

<210> 57

<211> 300

<212> DNA

<213> Human papillomavirus type 35

<400> 57  
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gacctatact gttatgagca attgtgtgac agctcagagg aggaggaaga tactattgac 120  
gggccagctg gacaagcaaa accagacacc tccaattata atattgtaac gtcctgttgt 180  
aaatgtgagg cgacactacg tctgtgtgta cagagcacac acattgacat acgtaaattg 240  
gaagatttat taatgggcac atttggaata gtgtgccccg gctgttcaca gagagcataa 300

<210> 58

<211> 330

<212> DNA

<213> Human papillomavirus type 39

<400> 58  
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atacagccgg ttgaccttgt atgtcacgag caattaggag agtcagagga tgaaatagat 120  
gaacccgacc atgcagttaa tcaccaacat caactactag ccagacggga tgaaccacag 180  
cgtcacacaa tacagtgttc gtgttgtaag tgtaacaaca cactgcagct ggtagtagaa 240  
gcctcacggg atactctgcy acaactacag cagctgttta tggactcact aggatttgtg 300  
tgtccgtggg gtgcaactgc aaaccagtaa 330

<210> 59  
<211> 321  
<212> DNA  
<213> Human papillomavirus type 45

<400> 59  
atgcatggac cccgggaaac actgcaagaa attgtattgc atttgaacc tcagaatgaa 60  
ttagatcctg ttgacctgtt gtgttacgag caattaagcg agtcagagga ggaaaacgat 120  
gaagcagatg gagttagtca tgcacaacta ccagcccgac gagccgaacc acagcgtcac 180  
aaaattttgt gtgtatgttg taagtgtgac ggcagaattg agcttacagt agagagctcg 240  
gcagaggacc ttagaacact acagcagctg tttttgagca ccttgtcctt tgtgtgtccg 300  
tggtgtgcaa ctaaccaata a 321

<210> 60  
<211> 306  
<212> DNA  
<213> Human papillomavirus type 51

<400> 60  
atgcgtggta atgtaccaca attaaaagat gtagtattgc atttaacacc acagactgaa 60  
attgacttgc aatgctacga gcaatttgac agctcagagg aggaggatga agtagataat 120  
atgcgtgacc agctaccaga aagacgggct ggacaggcta cgtgttacag aattgaagct 180  
ccgtgttgca ggtgttcaag tgtagtacaa ctggcagtgg aaagcagtgg agacaccctt 240  
cgcgtgttac agcagatggt aatgggcgaa ctaagcctgg tttgcccggtg ttgtgcgaac 300  
aactag 306

<210> 61  
<211> 300  
<212> DNA  
<213> Human papillomavirus type 52

<400> 61  
atgcgtggag acaaagcaac tataaaagat tatatatag atctgcaacc tgaaacaact 60  
gacctacact gctatgagca attaggtgac agctcagatg aggaggatac agatggtgtg 120  
gaccggccag atggacaagc agaacaagcc acaagcaatt actacattgt gacatattgt 180  
cacagttgtg atagcacact acggctatgc attcatagca ctgcgacgga ccttcgtact 240  
ctacagcaaa tgctgttggg cacattacaa gttgtgtgcc ccggctgtgc acggctataa 300

<210> 62  
<211> 318  
<212> DNA  
<213> Human papillomavirus type 56

<400> 62  
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attgacctac agtgcaatga gcaattggac agctcagagg atgaggatga ggatgaagta 120



gaccatttgc aggagcggcc acagcaagct agacaagcta aacaacatac gtgttaccta 180  
atacacgtac cttgttgtga gtgtaagttt gtggcgcagt tggacattca gagtacaaaa 240  
gaggacctgc gtgtgtgaca acagctgctt atgggtgcgt taacagtaac gtgcccactc 300  
tgcgcatcaa gtaactaa 318

<210> 63  
<211> 297  
<212> DNA  
<213> Human papillomavirus type 58

<400> 63  
atgagaggaa acaacccaac gctaagagaa tatatttttag atttacatcc tgaaccaact 60  
gacctattct gctatgagca attatgtgac agctcagacg aggatgaaat aggcttggac 120  
gggccagatg gacaagcaca accggccaca gctaattact acattgtaac ttgtttgttac 180  
acttgtggca ccacggttcg tttgtgtatc aacagtacaa caaccgacgt acgaacccta 240  
cagcagctgc ttatgggcac atgtaccatt gtgtgcccta gctgtgcaca gcaataa 297

<210> 64  
<211> 324  
<212> DNA  
<213> Human papillomavirus type 59

<400> 64  
atgcatggac caaaagcaac actttgtgac attgttttag atttgggaacc acaaaaattat 60  
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gatgaaccag atggagttaa tcatcctttg ctactagcta gacgagctga accacagcgt 180  
cacaacattg tgtgtgtgtg ttgtaagtgt aataatcaac ttcagctagt agtagaaacc 240  
tcgcaagacg gattgcgagc cttacagcag ctgtttatgg acacactatc ctttgtgtgt 300  
cctttgtgtg cagcaaacca gtaa 324

<210> 65  
<211> 333  
<212> DNA  
<213> Human papillomavirus type 68

<400> 65  
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atacagccgg ttgaccttgt atgtcacgag caattaggag attcagacga tgaaatagat 120  
gaaccgacc atgcagttaa tcaccaccaa catctactac tagccagacg ggacgaacaa 180  
cagcgtcaca gaattcagtg tctgtgttgt aagtgtaca aggcaactgca actagtagta 240  
gaagcgtcgc gggacaacct gcggacacta caacagctgt ttatggactc actaaatatt 300  
gtgtgtccgt ggtgtgcaac tgaaaccag taa 333

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